# Studies in Natural Products Chemistry

## Volume 30
## Bioactive Natural Products (Part K)

**Studies in Natural Products Chemistry**
**edited by Atta-ur-Rahman**

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Vol. 30 Bioactive Natural Products (Part K)

# Studies in
# Natural Products Chemistry

## Volume 30

## Bioactive Natural Products (Part K)

Edited by

**Atta-ur-Rahman**

*H.E.J. Research Institute of Chemistry,*
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# FOREWORD

The present volume, the $30^{th}$ of this series, continues to focus on bioactive natural products and contains articles written by authorities in their respective fields.

Chemistry and biological activities of discodermolide and pironetin, two immunosuppressive agents with antitumor activity, have been described by Cossy and coworker. Antitumor and vascular physiological effects of natural products are described in another interesting article by Kimura. A number of natural products have shown anti-obesity effects which have been discussed by Han and coworkers. The review by Tyman and co-worker presents the chemistry and pharmacological activity of phenolic lipids. The discovery of new analgesic drugs continues to be a challenge for medicinal chemists and natural materials have been explored widely in this respect. The review by Yunes and co-workers describes the use of natural products as sources of new analgesic drugs.

Biologically active polyphenols can be prepared by acid-treatment of arylpropanoid and arylethanoid derivatives. Cotelle has presented a comprehensive review of such reactions which can lead to numerous polyphenols exhibiting a wide variety of biological properties such as anti-inflammatory, anticancer and antiviral activities. The Turkish group led by Ulubelen and co-workers have presented a review on Labiatae flavonoids which describes the biological activities of a number of important and interesting compounds. Quinones constitute a structurally diverse class of phenolic compounds with a wide range of pharmacological properties. A review in this area by Martinez and co-workers presents the chemistry and pharmacological activities of such compounds. Autism is a pervasive developmental disorder characterized by impairments in communication, socialization and restricted stereotyped behavior and interests. A review on serotonin and the etiology of autism by Ruiz-Rubio and co-worker presents recent developments in this field.

Natural products continue to be explored for antiviral compounds. Martinez and co-workers present a review on antiviral activities of polysaccharides from natural sources which should prove to be interesting to medicinal chemists. 3-Deoxy-ulosonic acids represent an important family of complex monosaccharides which have diverse structures and properties in a wide range of biological processes. A review by Banaszek and co-worker describes the recent advances in the chemistry of such bioactive substances. A number of naturally occurring vitamin D analogues (vitamin $D_2$, $D_3$, $D_4$, $D_5$, $D_6$ and $D_7$) exhibit interesting biological activities. A review by Tachibana and co-worker on structure-activity relationships of naturally occurring active forms of vitamin 'D' analogues describes important developments in this field. Carotenoids constitute a class of bioactive natural products. Carotenoid cation radicals are involved in photosynthesis and in antioxidant actions. A review by Liaaen-Jensen and co-worker describes recent developments in this area. The genus *Hernandia* has proven to be a source of a number of compounds which have been found to exhibit various biological activities, such as antiplasmodial action, antiplapelet aggregation, cytotoxicity, inhibition of Epstein-Barr virus, etc. The biologically active constituents of this genus are discussed in detail by Kinghorn and co-worker. Plants of genus *Hypericum* have been used as traditional remedies in several parts of the world. St. John's Wort (*H. perforatum*) has been found to possess wound-healing and anti-inflammatory properties.

Avato presents a comprehensive view of secondary metabolites of this genus. Bioactive triterpenes and related compounds from Celastraceae are of interest due to the wide range of biological activities shown by those compounds. The isolated compounds showed mainly antimicrobial, cytotoxic, antitumoral, antiviral, anti-inflammatory, hepatoprotective, antifeedant and insecticidal activities. A review by Alvarenga and co-worker describes recent developments in this area. Biologically active substances from the genus *Baccharis* L. (Compositae) have been reviewed by the Spanish group comprising Martinez and co-workers. *Chlorella vulgaris (*strain CK) has been used as a health food for the past 30 years in Japan and in other countries. This strain has been shown to exhibit a number of pharmacological effects in animals and humans. A review of recent developments in this field is presented by Hasegawa and co-workers. Endogenous opioids have been studied extensively since their discovery in the hope of finding a perfect analgesic. However, the design of such substances has proven to be very challenging. A review on the pharmacological activities of new receptor agonists and antagonists is presented by Capasso and co-worker. Earthworm fibrinolytic enzymes (EFEs) are a group of fibrinolytic enzymes, secreted by the alimentary tract of earthworm. Their current clinical utilization and some potential new applications are discussed by Zhao and co-workers.

The present volume, volume 30 of this series, contains 963 pages and is accompanied with an independent comprehensive volume of indices. The index volume comprising 1376 pages, contains the following four indices:

a) Cumulative Biological Source Index
b) Cumulative Organic Synthesis Index
c) Cumulative Pharmacological Activity Index
d) Cumulative General Subject Index

This should allow the readers to extract the relevant information from any of the first 30 volumes of the series efficiently.

The material in this volume should be of interest to a large number of organic chemists, medicinal chemists, pharmaceutical chemists and others involved in new drug development. I hope that it will be received with the same excitement and interest as the previous volumes of the series.

I would like to express my thanks to Mr. Liaquat Raza and Ms Qurat-ul-Ain Fatima for their assistance in the preparation of the index. I am also grateful to Mr. Waseem Ahmad for typing and to Mr. Mahmood Alam for secretarial assistance.

**Atta-ur-Rahman**
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September, 2004

# PREFACE

This the 30$^{th}$ volume in the series, "Studies in Natural Products Chemistry", is the 21$^{st}$ in a long collection devoted to the biological aspects of natural products. The study of natural products leads to an ever-increasing source of new, natural medicinal materials and also provides a fountain of new ideas for medicinal and other synthetic chemists to design new and unnatural drugs whose structures are inspired by the natural materials themselves.

Yet again the scientific community owes a large debt of gratitude to Professor Atta-ur-Rahman, who is both Director of the H.E.J. Research Institute of Chemistry at the University of Karachi as well as the Federal Minister of Higher Education in Pakistan for once again overseeing the selection of a distinguished group of authors to discuss recent major advances in the field and for bringing their endeavors to its present splendid final form. His dedication of time and energy to this important undertaking is of great value to the scientific community at large and to the public in general. Many useful therapeutic agents are being derived from the ever-increasing number of applications of natural product studies to modern health care.

Alan R. Katritzky, FRS
Kennan Professor of Chemistry
University of Florida

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# Bioactive Natural Products

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# SYNTHETIC ROUTES TO DISCODERMOLIDE AND PIRONETIN: TWO IMMUNOSUPPRESSIVE AND ANTITUMORAL AGENTS

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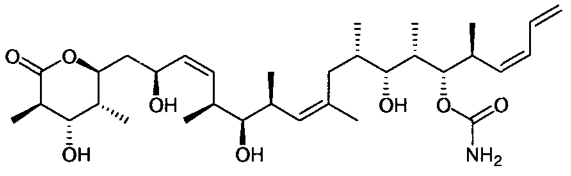
**ABSTRACT**: Discodermolide and pironetin are two immunosuppressive agents with antitumor activity. The isolation, structure elucidation, biological activity are reported as well as the synthetic routes to these two compounds. Five syntheses of discodermolide and seven syntheses of pironetin are described.

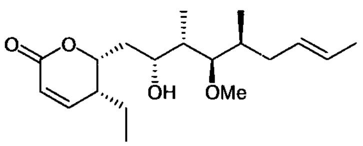
## INTRODUCTION

Recently, it has been noticed that natural products with polyketide units can be potent immunosuppressive agents as well as antitumor agents. Two structurally different polyketide lactones, discodermolide and pironetin belong to this group of compounds (Fig. 1). Due to the potential therapeutic applications and the extreme scarcity of discodermolide and pironetin, interest in the chemical synthesis of these two natural products and in their analogues continues unabated.

Figure 1




(+)-Discodermolide




( )-Pironetin

## Discodermolide

### *Isolation, Structure Elucidation and Biological Activity*

Discodermolide is a unique polyketide isolated in 1990 by Gunasekera et al. at the Harbor Branch Oceanographic Institute from the Caribbean deep sea sponge *Discodermia dissoluta* [1-2]. Methanol/toluene (3:1) solvent extraction of the sponge yielded an extract that was partitioned between ethyl acetate and water. By silica gel and reverse-phase chromatography, (+)-discodermolide was purified from the ethyl acetate-soluble material and obtained as crystalline solid in 0.002% yield (w/w from frozen sponge).

Its structure was determined by extensive spectroscopic studies, including a combination of 1D and 2D NMR techniques. The relative stereochemistry was assigned by single crystal X-ray crystallography [1]. The absolute configuration however remained undefined until Schreiber and co-workers [3] synthesized both antipodes. Discodermolide is a 24-membered polyketide carbon skeleton with thirteen stereogenic centers, a tetrasubstituted δ-lactone (C1-C5), one disusbtituted and one trisubstituted (*Z*)-double bond, a pendant carbamate moiety at $C_{19}$ and a terminal (*Z*)-diene (C21-C24). In the solid state, discodermolide adopts a U-shaped conformation, where the two (*Z*)-olefins in the side chain act as conformational locks by minimizing $A_{1,3}$ strain between their respective substituents in concert with the avoidance of *syn*-pentane interactions. The tetrasubstituted δ-lactone prefers a boat like conformation. (+)-Discodermolide appears to be made up of eight propionate and four acetate units. It is likely that this dodecaketide is biosynthetized by a symbiotic microorganism associated with *Discodermia dissoluta* species, involving a modular polyketide synthase.

Initial biological studies revealed that (+)-discodermolide suppresses both the two-way mixed-lymphocyte reaction and the concanavalin A induced mitogenesis of murine splenocytes *in vitro* ($IC_{50}$ 0.24 nM and 0.19 nM respectively) with no associated cytotoxicity [4]. (+)-Discodermolide also inhibits the graft *versus* host-splenomegaly response induced by injection of parental splenocytes into F1 recipient mice, with potency intermediate between those of cyclosporin A and FK 506 [5]. These findings stimulated considerable interest in discodermolide as a possible immunosuppressant.

Subsequently, (+)-discodermolide was found to be a potent cell growth inhibitory agent, which arrests cell development at the M phase by binding and stabilizing mitotic spindle microtubules [6]. Thus, discodermolide joins the group of antimitotic agents to act by microtubule stabilization. Among the microtubule stabilizing agents, taxol [7], epothilone [8], eleutherobin [9], laulimalide [10], discodermolide has been recognized as one of the most potent tubulin polymerizing agents presently known. Despite having no apparent structural similarities, discodermolide has been found to stabilize microtubules more potently than taxol and competitively inhibits its binding to tubulin polymers [4-11]. The growth of taxol-resistant ovarian and colon cells is <2.5nM [12], while the timing and type of DNA fragmentation induced is consistent with the induction of apoptosis [13]. In recent studies, it was found that discodermolide was unable to act as a substitute for taxol [14], and that low concentrations of taxol amplified the cytotoxicity of discodermolide 20-fold against the human lung carcinoma cell line (A549-T12) [9-15]. The biological profile of discodermolide makes it a promising candidate for clinical development as a chemotherapeutic agent for taxol-resistant breast, ovarian and colon cancer and other multi-drug resistant cancers. It has been noticed that the (-)-antipode was a potent inhibitor of cell proliferation that the full length of discodermolide is required for recognition by its receptor and that the conformation about C16 and C17 is critical to the activity. Hovewer, the presence of the methyl at C16 is not crucial. 16-Desmethyl as well as the α- and β-thioanomers were equally active *in vivo* in inhibiting proliferation as (+)-discodermolide [4].

***Synthetic Routes to Discodermolide***

Clinical development is hampered due to the extremely scarce supply of discodermolide from the natural source. Thus, total synthesis provides the only viable route to useful quantities of this novel cytotoxic polyketide. Consequently, there have been considerable synthetic efforts toward the total synthesis of discodermolide [16]. Up to date, five total synthesis of (+)- and/or (-)-discodermolide have been reported, one by Schreiber [3], one by Smith [17], one by Myles [18], one by Marshall [19] and one by Paterson [20]. All the syntheses are convergent. Schreiber and Myles employed an analogous connection by using a Nozaki-Kishi addition of a C8 acetylenic or (*Z*)-vinylic chromium to a C7 aldehyde for the major

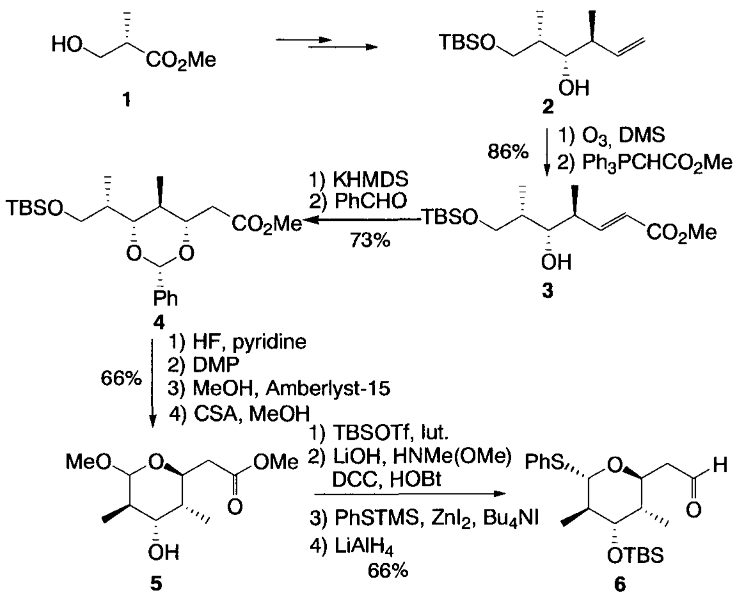
coupling event. Both Marshall and Smith utilized as the major coupling event, a C9-C14 vinyl iodide with a C15-C21 iodozinc reagent in a palladium catalyzed coupling reaction. Paterson [20] uses aldol reactions between an enolate of a C16 ester to a C17 aldehyde and an enolate addition of a C6 ketone to a C7 aldehyde.

*Schreiber's Synthesis [3]*

Schreiber et al. have described the first synthesis of (+)-discodermolide and of its (-)-antipode by using the same strategy. The synthesis of (+)-discodermolide was achieved by the coupling of three fragments **6**, **8**, **11** of approximately equal complexity (Schema 1, 2, 3) which were synthesized from (*S*)-(+)-3-hydroxy-2-methylpropionate **1** by using Roush's chiral boronates, allowing the control of nine of discodermolide's thirteen stereocenters. All the other stereocenters were set by substrate-controlled reactions.

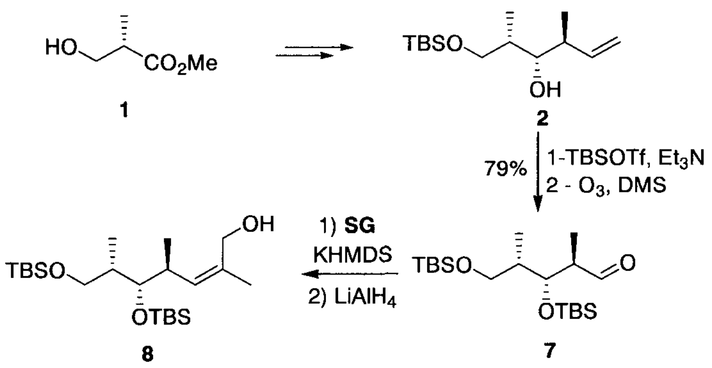
The synthesis of the C1-C7 fragment, which corresponds to the lactone, starts with the homoallylic alcohol **2** which was prepared from **1**. The existing stereocenter and the conjugate addition method of Evans [21] allow the control of the C5 stereocenter. The homoallylic alcohol **2** was oxidatively cleaved and homologated to the *trans* enoate **3** by a Wittig olefination. Treatment of **3** with benzaldehyde and a catalytic amount of KHMDS provided acetal **4**. The internal Michael addition of the hemiacetal intermediate proceeds with complete stereoselectivity [22]. After deprotection and oxidation, the corresponding aldehyde was treated with Amberlyst-15 and then with camphor sulfonic acid (CSA), to yield pyrane **5** as a mixture of β- and α-anomers (1.8/1). This compound was converted to the thiophenyl acetal **6** (4 steps) as this compound can be hydrolyzed later under mild conditions ($Hg^{2+}$) with subsequent oxidation of the lactol to the desired lactone. Compound **6** represents the C1-C7 fragment of discodermolide (Scheme 1).

Scheme 1




The major challenge in the synthesis of the C9-C15 fragment is the preparation of the trisubstituted olefin with stereochemical control. Homoallylic alcohol **2** was protected and oxidatively cleaved to provide an intermediate aldehyde **7** which was treated with the Still-Gennari reagent [$(CF_3CH_2O)POCH(Me)CO_2Me$ = **SG**] [23] to produced the *cis*-trisubstituted olefin. Reduction of the resulting enoate provided allylic alcohol **8** which will be then coupled with the C1-C7 fragment and then with the C16-C24 fragment (Scheme 2).

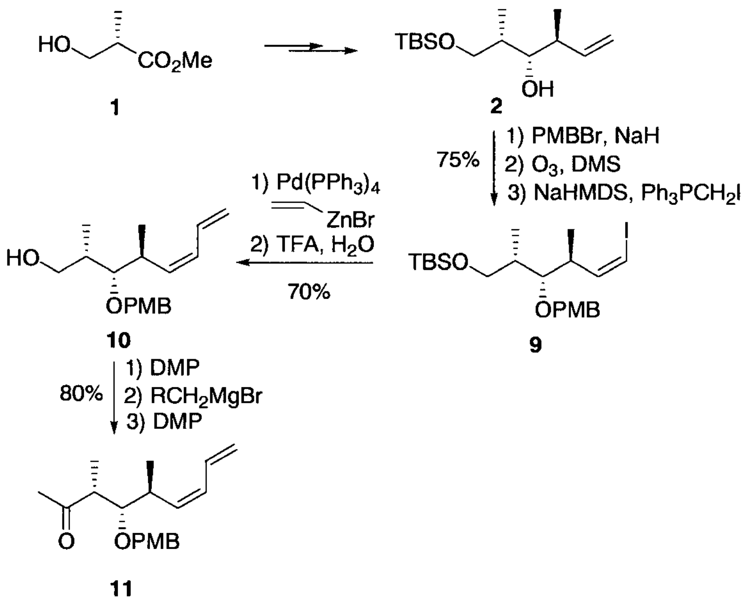
Scheme 2




$(CF_3CH_2O)_2POCH(Me)CO_2Me$ = **SG**

The C16-C24 ketone fragment **11** was prepared from the homoallylic alcohol **2**. After protection of **2** as a *p*-methoxybenzyl (PMB) ether [24], the oxidative cleavage led to an intermediate aldehyde which was converted to the *cis* vinyl iodide **9** by using the (iodomethylene)triphenylphosphorane [25]. The vinyl iodide was converted to diene **10** by a palladium-catalyzed coupling reaction with vinylzinc bromide [26]. After deprotection, oxidation (Dess-Martin periodinane) and subsequent Grignard addition of methylmagnesium bromide, a 4/1 mixture of epimeric alcohols was obtained and then oxidized (Dess-Martin periodinane = DMP) to ketone **11** (Scheme 3).

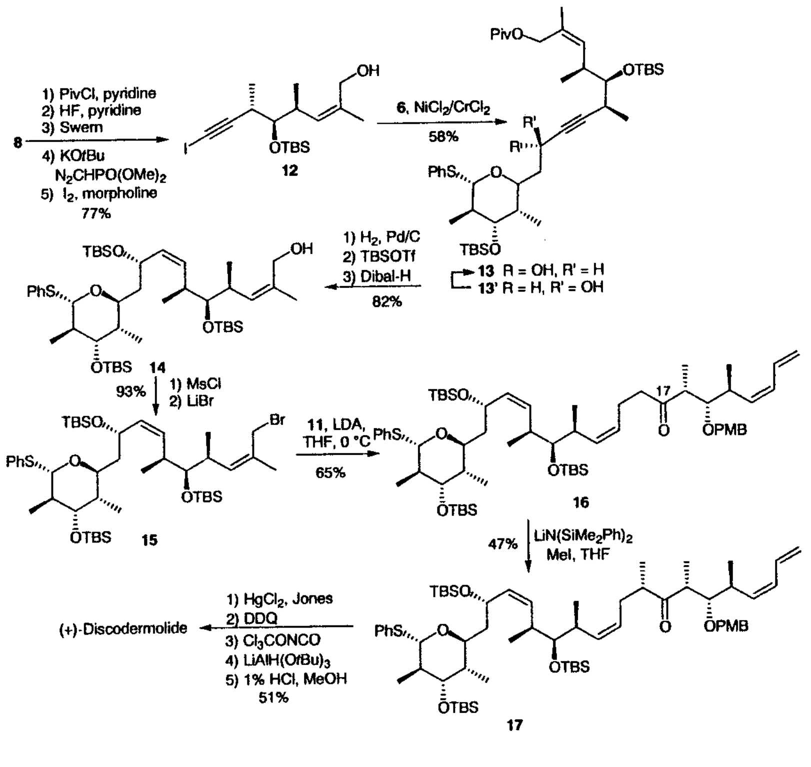
Scheme 3




Different approaches to achieve the coupling of the three fragments were tried. The best coupling started by a Nozaki-Kishi reaction between aldehyde **6** and the C8-C15 acetylenic fragment **12** which comes from the five-step transformation of **8** (Scheme 4). Coupling of the iodoacetylene **12** with aldehyde **6** under Nozaki-Kishi conditions yielded two epimers **13** and **13'** in a 2:1 mixture [27]. The minor isomer **13'** was recycled by using a Swern oxidation and the obtained ketone was reduced by Corey's asymmetric reduction protocol by using catecholborane and a catalytic amount of *B*-butyloxazaborolidine derived

from D-proline [28]. Semi-hydrogenation of **13** with 10% Pd/C, TBS protection, removal of the pivaloyl group with Dibal-H afforded allylic alcohol **14**. Conversion of **14** to bromide **15** and its coupling with ketone **11** (LDA) afforded the full carbon backbone of discodermolide. Alkylation of the *Z*-enolate derived from **16** [$LiN(SiMe_2Ph)_2$, 0 °C] with methyl iodide provides the correct desired stereochemistry at C16. The final challenges in the synthesis of (+)-discodermolide was the stereoselective reduction of the C17 ketone and the selective carbamoylation of the C19 alcohol. The final elaboration to (+)-discodermolide proceeded by the transformation of the thiophenyl acetal to the lactone, then DDQ deprotection of the PMB ether, carbamoylation and reduction of the ketone at C17 by using the bulky $LiAlH(OtBu)_3$, which led to the desired epimer in a ratio 30/1 presumably due to the neighbouring carbamate that may facilitate a chelation-controlled reduction and cleavage of the silyl ether by using 1% HCl in MeOH (Scheme 4).

Scheme 4



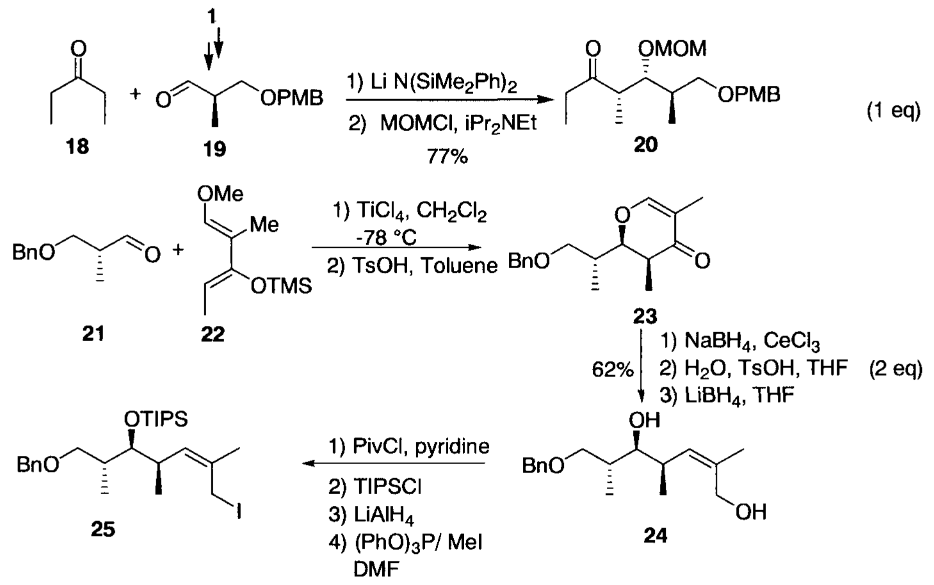
A similar strategy was employed for synthesizing (-)-discodermolide starting from (*R*)-(3)-hydroxy-2-methyl propionate **1**'[3b], synthetic variant of the natural product were also prepared such as 16-*epi*-discodermolide, truncated versions of discodermolide were also synthesized such as the C1-C15, C16-C24 and C8-C24 fragments.

*Myles's Synthesis of Discodermolide [18, 29, 30]*

Myles et al. have synthesized the antipole of the natural discodermolide, but their strategy can be applied to the synthesis of (+)-discodermolide. They have adopted a convergent strategy to synthesize discodermolide, disconnecting the C7-C8 and C15-C16 bonds. In this synthesis, the key steps are two coupling reactions. One is a diastereoselective alkylation between an ethylketone and an allylic iodide to form the C15-C16 bond, and the other coupling reaction is a metal-promoted coupling reaction of a (*Z*)-vinyl chromium derivative with an aldehyde to form the C8-C9 bond by utilizing a Nozaki-Kishi reaction.

As previously, (*S*)-(+)-3-hydroxy-2-methylpropionate **1** is the starting compound for the synthesis of (-)-discodermolide and allowed the synthesis of ethyl ketone **20** (Scheme 5, eq. 1) [29] and vinyl iodide **25** (Scheme 5, eq. 2) [30]. Ethyl ketone **20** was prepared directly by a diastereoselective aldol condensation of the lithium anion of 3-pentanone **18** and (*R*)-3-*p*-methoxybenzyloxy-2-methylpropionaldehyde **19** [31], followed by the protection of the resulting alcohol as a methoxymethyl ether, leading to **20** which corresponds to the C16-C21 fragment. The selection of the methoxymethyl group for protection of the alcohol beta to the ketone was expected to facilitate the introduction of the C19 carbamate, function at a late stage of the synthesis. Allyl iodide **25** was obtained from aldehyde **21** which was also synthesized from (*S*)-(+)-3-hydroxy-2-ethylpropionate **1**' [32,33]. After the cyclocondensation of diene **22** with aldehyde **21** and treatment with TsOH, dihydropyranone **23** was isolated. This later compound serves as the template for the establishment of the required *Z*-trisubstituted alkene of discodermolide. After reduction, acidic treatment and reduction of the pyrane ring ($LiBH_4$), the desired *Z*-allylic alcohol **24** was isolated and transformed in four steps to the allyl iodide **25** (Scheme 5, eq. 2).

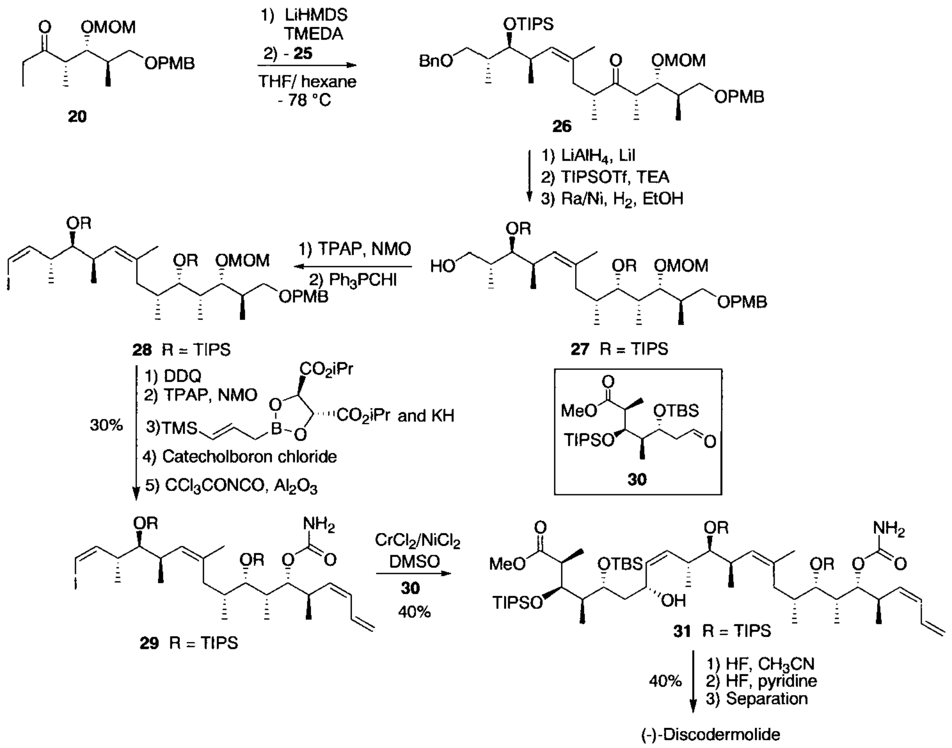
Scheme 5



Treatment of **20** with LiHMDS and tetramethylethylene diamine in THF at -75 °C followed by the addition of allyl iodide **25** led to a smooth chelation-controlled alkylation reaction and the desired diastereomer **26** was obtained along with the C16 diastereomer in a ratio of 6/1 (70% yield). After a chelation-controlled reduction of ketone **26** with LAH in the presence of lithium iodide, the corresponding alcohol at C17 was obtained with good diastereoselectivity (dr = 8/1). Protection of the secondary alcohol and hydrogenolysis of the C9-benzyl ether (Raney Nickel/$H_2$) afforded the C9-C21 synthon **27** (71% yield) and facilitated the separation of all the minor diastereomers. A Nozaki-Kishi coupling reaction between the *Z*-vinyl iodide **29** and aldehyde **30** [34] was the best option to prepare the carbon skeleton of discodermolide. To prepare the required vinyl iodide **28**, with a good *Z*/*E* selectivity (20/1), alcohol **27** was oxidized (TPAP) and treated immediately with iodomethylene-triphenylphosphorane [25]. After cleavage of the PMB protecting group at C21 by using DDQ, the obtained alcohol was oxidized to the aldehyde and this compound was transformed to a *Z*-diene by using a modified Peterson olefination [35] (*Z*/*E*: 20/1) (70% yield). After hydrolysis of the methoxymethyl ether (catecholboron chloride), the free alcohol at C21 was obtained and transformed to the carbamate (trichloroacetyl

isocyanate) [36]. The transition metal ($NiCl_2$, $CrCl_2$, DMSO) promoted addition of the vinyl iodide to aldehyde **30** [37] afforded the C1-C14 fragment of discodermolide **31** in 40% yield as a mixture of epimers at C7 (dr = 2.5/1). The removal of the two protecting silyl groups and lactonization were achieved by using 10% HF in $CH_3CN$ and the last protecting ether group was removed by HF/pyridine. At this stage (-)-discodermolide and the C7-*epi*-discodermolide could be separated. This highly convergent synthesis of discodermolide takes advantage of a chelation-controlled alkylation reaction to achieve a highly selective coupling reaction (Scheme 6).

Scheme 6



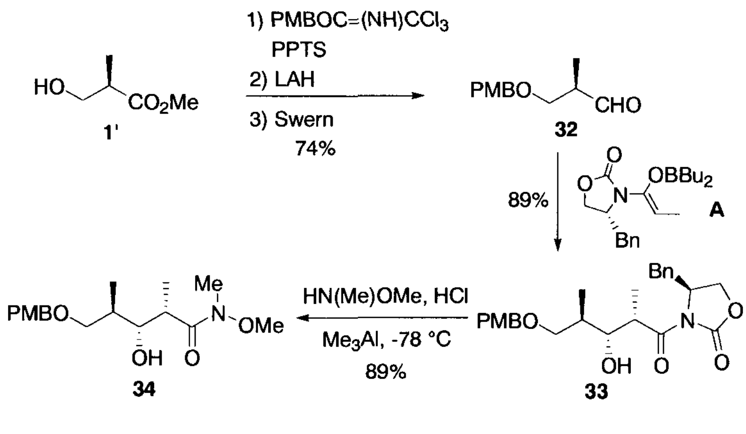

The two following syntheses of discodermolide by Smith and by Marshall joined a C9-C14 vinyl iodide with a C15-C21 iodozinc reagent in a palladium coupling reaction.

*Smith's Syntheses [17]*

In 1995 [17a], Smith et al. reported a highly convergent total synthesis of (-)-discodermolide by using Evans *syn* aldol reactions to control the stereogenic centers and a Negishi coupling reaction [38] to join the fragments C9-C14 and C15-C21. In their syntheses, Smith et al. took also advantage of a repeating stereotriad of continuous stereocenters that are separated by a (*Z*)-olefinic linkage at C8-C9 and C13-C14 and they have, as Schreiber, disconnected discodermolide in three fragments **36**, **38**, **42**, (Scheme 8) each of them were synthesized from a common precursor **34** (Scheme 7). The union of the fragments at the olefinic linkages and the introduction of the terminal diene moiety exploited a combination of stereocontrolled σ- and π-bond constructions.

As the role of the common precursor **34** is critical, it has been synthesized in large scale by using an Evans *syn* aldol protocol [39], starting from the commercially available (*R*)-(-)-methyl 3-hydroxy-2-methyl propionate **1'**. This compound was transformed in three steps to aldehyde **32** [40, 41] and used in an Evans aldol reaction with the dibutylboron enolate **A**. The Weinreb amide formation completed the construction of the common precursor **34** [42] (Scheme 7). From this precursor fragments C1-C8, C9-C14 and C15-C21 were synthesized (Scheme 8).

Scheme 7



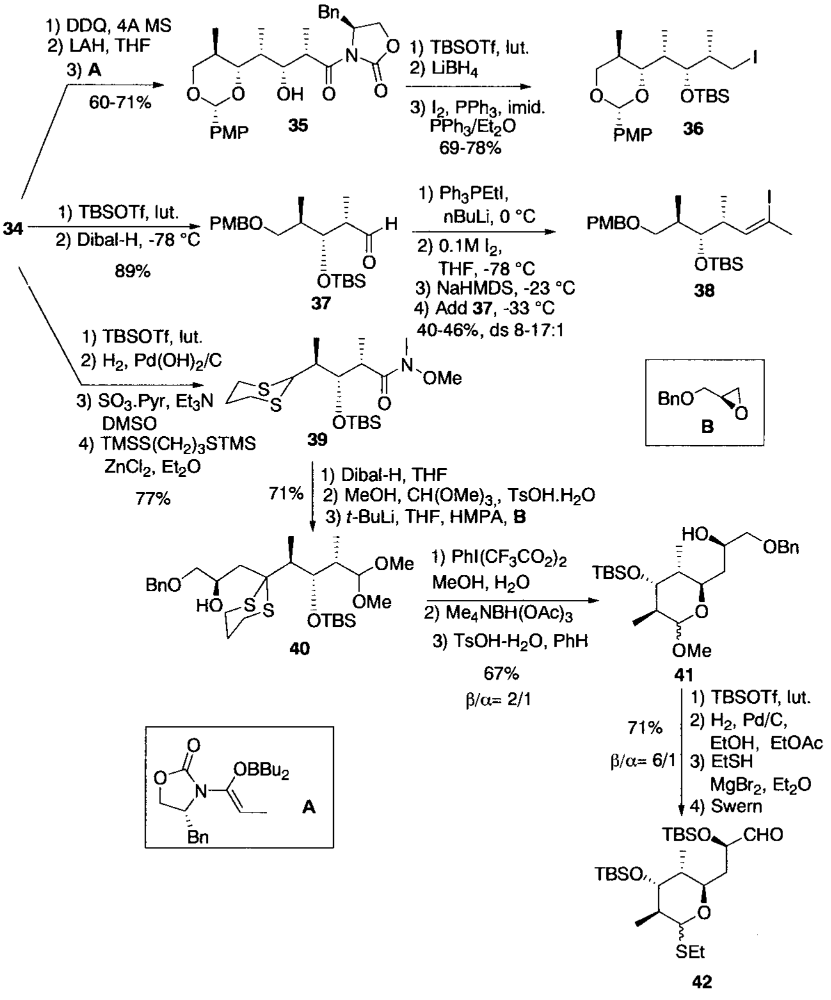

Compound **34** was transformed to fragment $C_{15}$-$C_{21}$ in six steps. The polyketide structural motif of this fragment suggested a second asymmetric aldol reaction. Compound **34** was transformed to the

*p*-methoxybenzylidene acetal [43] which facilitated the selective introduction of the terminal diene and of the carbamate moiety at the late-stage. After treatment of **34** with DDQ and reduction of the Weinreb amide, the aldehyde was obtained and was directly coupled with the dibutylboron enolate **A** *via* an Evans aldol reaction to provide compound **35** with the requisite five contiguous stereogenic centers. After protection of the secondary hydroxyl group (TBSOTf), removal of the chiral auxiliary by reduction ($LiBH_4$) [44], the obtained alcohol was converted to iodide **36** [45]. This compound was obtained with an overall yield of 56% for the six steps (Scheme 8).

Compound **34** was transformed to vinyl iodide **38**, which corresponds to the C9-C14 fragment of discodermolide. After protection and reduction (Dibal-H), **34** was transformed to aldehyde **37** which was then converted to the (*Z*)-vinyl iodide **38** by using the iodoethylphosphonium ($EtPPh_3I$) [46]. The overall yield for the preparation for vinyl iodide **38** from **34** is 35% (Scheme 8).

To prepare the C1-C8 fragment from **34**, Smith et al. used a stereospecific generation of protected aldol linkages *via* dithiane-epoxide coupling [47]. The synthesis began with the protection of the hydroxy group in **34** (TBSOTf) followed by the removal of the PMB group [$Pd(OH)_2$; $H_2$], oxidation with $SO_3$.Pyr [48] and conversion to dithiane **39** [49]. Dibal-H reduction, formation of the dimethoxy acetal and addition of the dithiane anion to benzyl (*R*)-(-)-glycidyl ether **B** furnished **40**. Dithiane cleavage [50], Evans directed reduction [$Me_4NBH(OAc)_3$] [51], and chemoselective cyclization led to methyl pyranosides (mixture $\beta/\alpha$: 2/1) which after a silylation, debenzylation, ethyl thioacetal formation and Swern oxidation were transformed to **42** (Scheme 8).

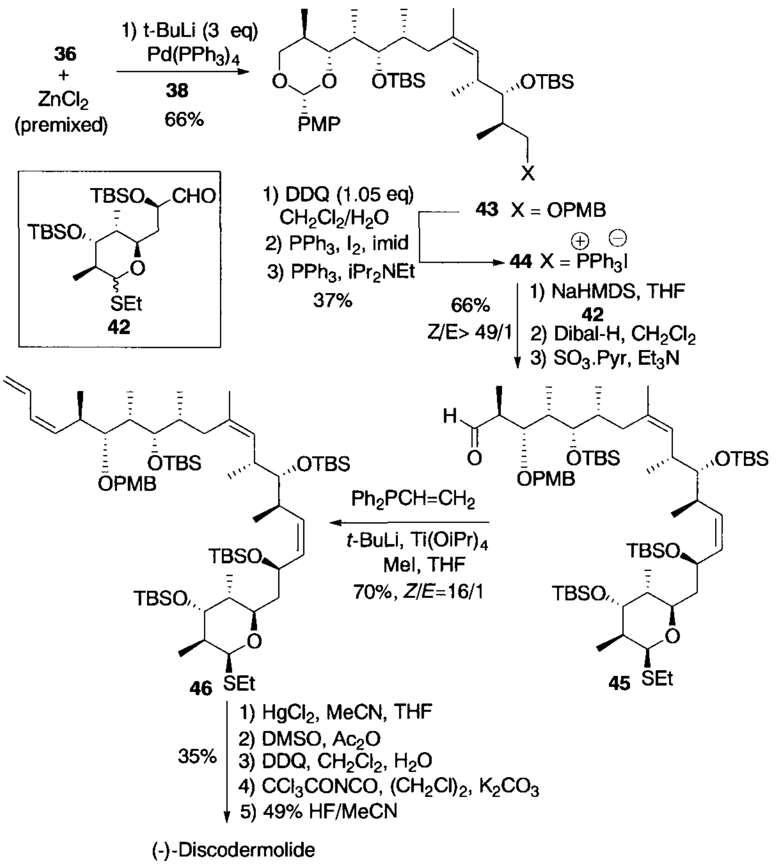
Scheme 8




With fragments **36**, **38** and **42** in hand, Smith et al. turned to the assembly of the discodermolide backbone. Reaction of vinyl iodide **38** with the organozinc derived from **36** [38, 52] in the presence of $Pd(PPh_3)_4$ [52] afforded **43** (66% yield). After selective removal of the PMB group, the resulting alcohol was transformed to the Wittig reagent **44** *via* an unstable iodide (Scheme 9). The union of **42** with the ylide derived from **44** and reductive opening of the acetal ring gave an alcohol, which was oxidized ($SO_3$.Pyr) to the corresponding aldehyde **45**. To complete the discodermolide backbone, a Yamamoto olefination [53] was applied to

the previously synthesized aldehyde and the *Z*-diene **46** was generated. Completion of the (-)-discodermolide was achieved after removal of the PMB group (DDQ), the introduction of the carbamate [36] and a final deprotection step (HF, $CH_3CN$) (Scheme 9).

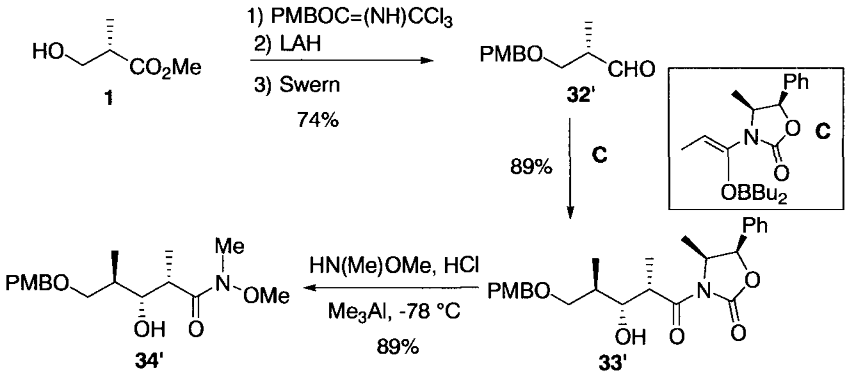
Scheme 9




In 1999 [17b], a highly efficient second generation synthesis of (+)-discodermolide was achieved on a one gram scale, maintaining the triply convergent approach utilized in the first-generation synthesis. However, notable improvements have been achieved in the synthesis of the C1-C8 fragment, obtained as an aldehyde-lactone unit, which greatly simplify the end-game of the synthesis and facilitate material throughput for a large scale. An improvment has been made also in the preparation of iodide **43**, by using ultrahigh pressure in a buffered non-polar medium

[54]. The synthesis of (+)-discodermolide was achieved from **34'**, which was prepared from (*S*)-(+)-methyl 3-hydroxy-2-methylpropionate **1** (Scheme 10). A slight modification has been made for practical reasons, the dibutylboron enolate **C** was used instead of the dibutylboron enolate **A** (Scheme 10). Compound **34'** was transformed to **36'** and **38'** by using the same pathway as for the synthesis of **36** and **38** (Scheme 11).

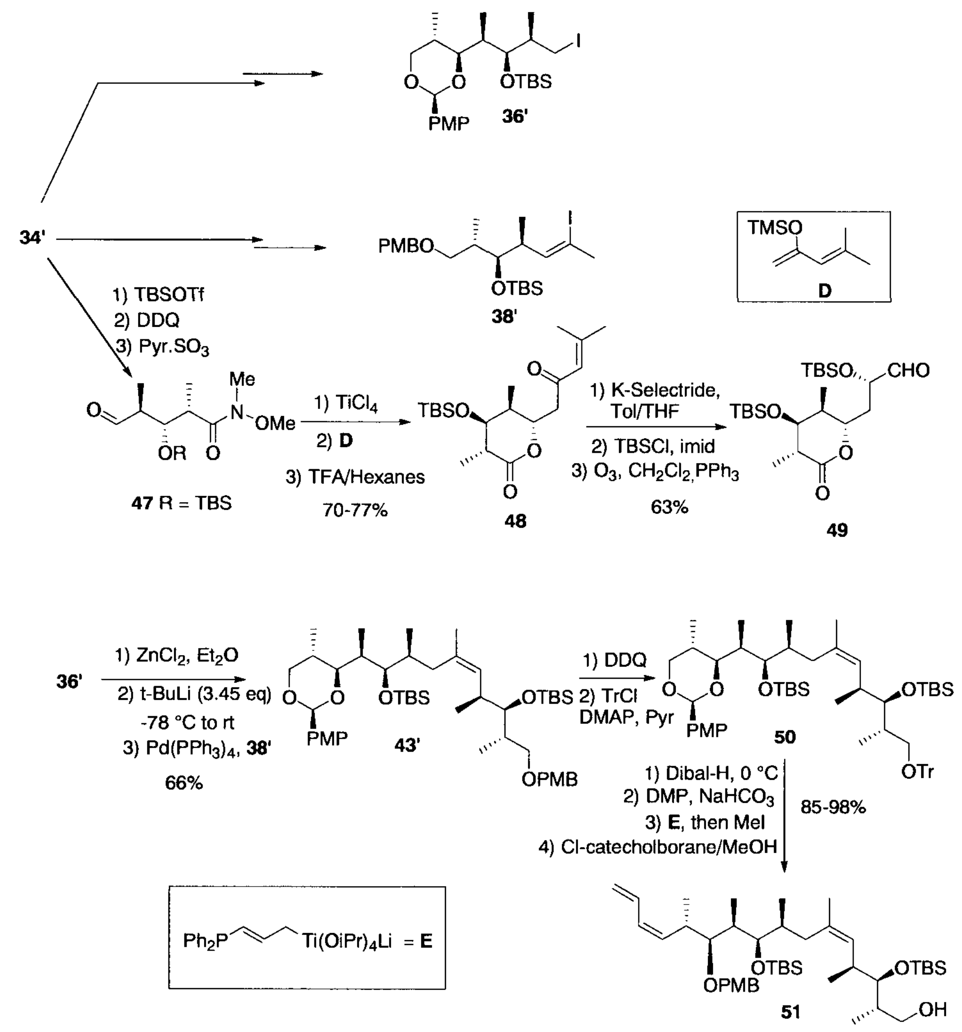
Scheme 10




To prepare the new C1-C8 fragment, the point of departure is compound **34'**. After silylation (TBSOTf), removal of the PMB group by hydrogenolysis and oxidation ($SO_3$.Pyr), the aldehyde **47** was obtained. Addition of the silylenol ether **D** to a premixed solution of aldehyde **47** and $TiCl_4$ afforded lactone **48** after acid-catalyzed (TFA) lactonization of the corresponding hydroxy-amide. Importantly, the Mukaiyama aldol reaction proceeded with a 20/1 selectivity favoring the *anti*-Felkin product. Reduction of enone **48** (K-Selectride, dr = 9/1), silylation of the hydroxy group and oxidative cleavage of the trisubstituted alkene ($O_3$, $PPh_3$) completed the synthesis of lactone **49**. The union of these three fragments **36'**, **38'** and **49** started as previously, with the palladium-catalyzed cross-coupling reaction of fragments **36'** and **38'**, leading to **43'**. At this point, the strategy is different from the previous one as the introduction of the diene moiety was achieved at this stage. To discriminate the protecting groups at C9 and C19, the PMB ether was removed chemoselectively (DDQ) [17a] and replaced by a trityl ether to afford **50**. Reductive opening of the acetal (Dibal-H) [55] liberated a primary alcohol which was oxidized (DMP) [56] and submitted to the allyltitanium phosphorus reagent **E** to produce a mixture of dienes in a ratio *E*/*Z*=12/1 from which the *Z* isomer can be isolated. After removal of

the trityl group [57], **51** was isolated in excellent overall yield (98%) (Scheme 11).

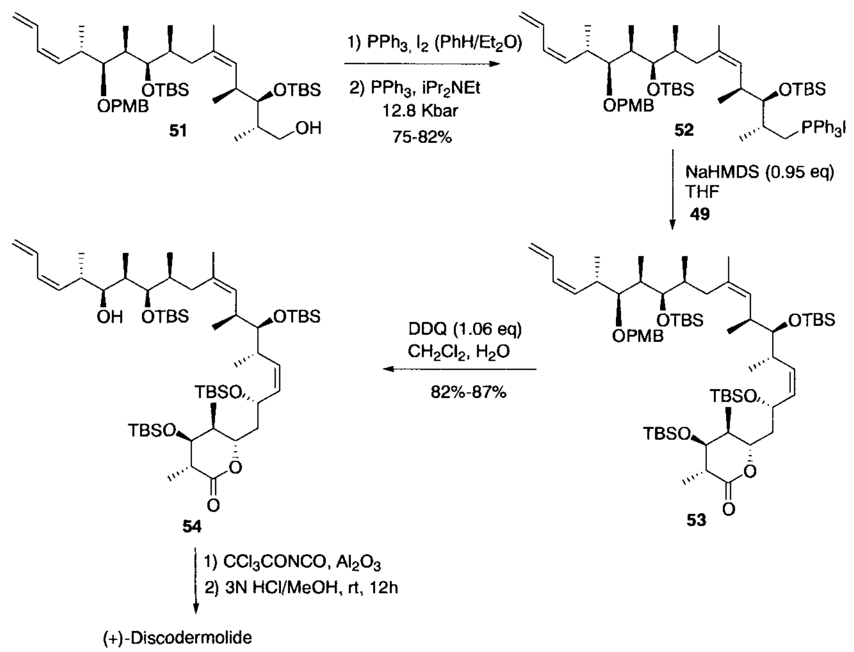
Scheme 11




The union of **51** with aldehyde **49** was achieved *via* the phosphonium iodide **52**, prepared from alcohol **51** under very peculiar conditions ($PPh_3$, $I_2$; then $PPh_3$ at ultrahigh pressure [54] in buffered non-polar medium). Addition of the phosphonium salt **52** to aldehyde **49** afforded the coupling product **53** in good yield and with an excellent *Z*/*E* selectivity (24/1). This compound represents the discodermolide carbon

skeleton. To complete the synthesis, **53** was treated with DDQ, the carbamate was installated ($CCl_3CONCO$, $Al_2O_3$) [36] and final deprotection leads to (+)-discodermolide (Scheme 12).

Scheme 12



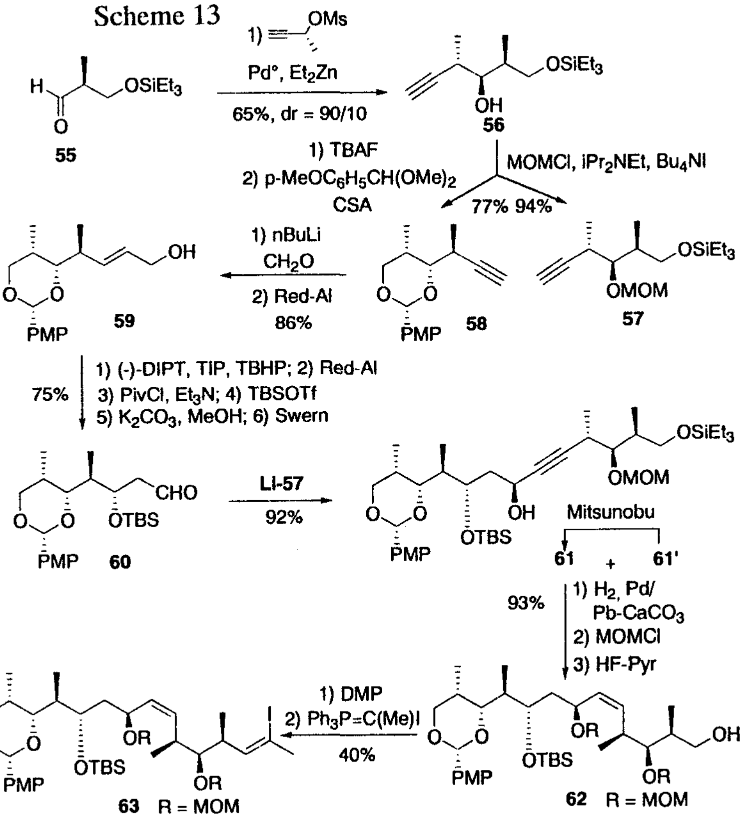

This synthesis proceeds in 6% overall yield and is amenable to gram-scale production.

*Marshall's Synthesis [19]*

Marshall's (+)-discodermolide approach involves assemblage of two subunits synthesized by addition of non-racemic allenyltin, allenylindium, and allenylzinc reagents to (*S*)-3-silyloxy-2-methylpropanal derivatives. A Suzuki coupling reaction between a C1-C14 boranate fragment and a C15-C24 vinyl iodide fragment led to the discodermolide skeleton. The synthesis of (+)-discodermolide begins with the addition of an allenylzinc, prepared *in situ* by the treatment of a propargylic mesylate with a catalytic amount of $Pd(PPh_3)_4$ and an excess of $Et_2Zn$ [58] to (*S*)-3-silyloxy-2-methylpropanol **55**. This addition afforded adduct **56** as a 90/10 mixture of separable diastereomers (65% yield). The obtained

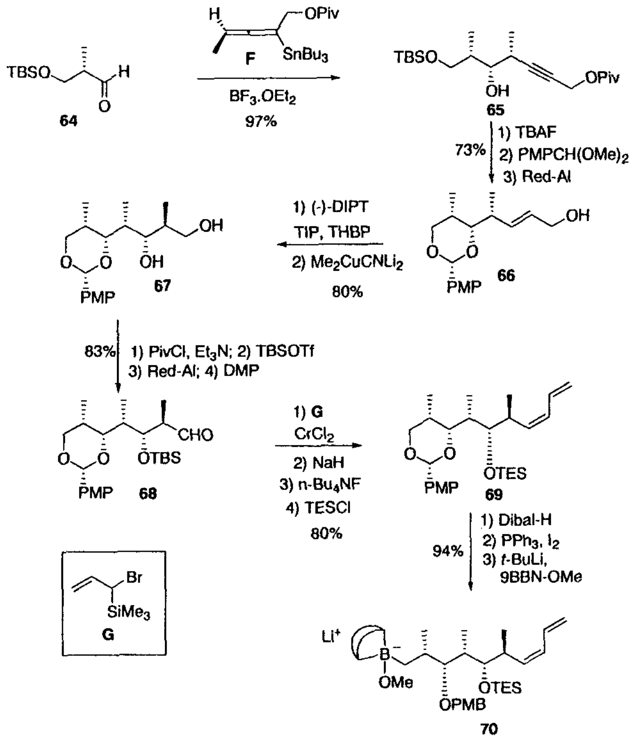
alcohol **56** was converted to the MOM derivative **57** (MOMCl, $iPr_2NEt$, $Bu_4NI$, 94% yield). The alcohol **56** was converted also to aldehyde **60** in ten steps. After deprotection-protection of **56**, the obtained acetylenic **58** was formylated, and the propargylic alcohol was subsequently reduced to allylic alcohol **59** [59]. Transformation of the allylic alcohol **59** to aldehyde **60** was accomplished by using a Sharpless epoxidation and subsequent reduction with Red-Al followed by a three-step protection-deprotection sequence and a Swern oxidation. Aldehyde **60** was then condensed with the lithiated alkyne **Li-57** to produce alcohols **61** and **61'** as a separable 85:15 mixture (92%). The minor isomer **61'** was inverted by a Mitsunobu reaction to produce **61**. The propargylic alcohol **61** was converted to the (*Z*)-allylic alcohol **62** by hydrogenation over Lindlar's catalyst, thus completing the assemblage of the C1-C13 backbone of (+)-discodermolide. This compound was transformed to the (*Z*)-vinyl iodide **63** after oxidation and condensation of the obtained aldehyde with the α-iodo ethylidene triphenylphosphorane [46]. Vinyl iodide **63** corresponds to the C8-C13 subunit (Scheme 13).

Scheme 13



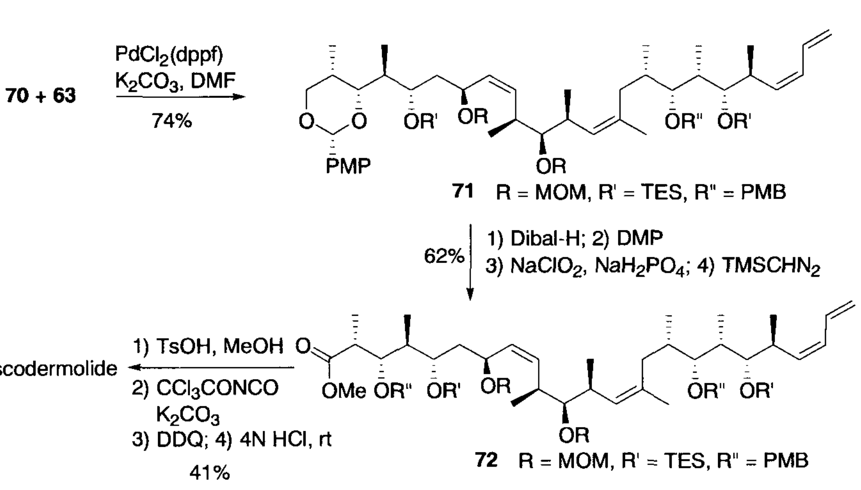
The second subunit to be prepared was the C15-C24 fragment. The synthesis of this fragment begins with the addition of an allenylstannane **F**[60] to the protected aldehyde **64** in a $S_E2$'addition promoted by $BF_3.OEt_2$ that produces the *syn,syn* adduct **65** (97% yield). After deprotection-protection and selective reduction (Red-Al), the allylic alcohol **66** was obtained. The stereogenic centers at C19 and C20 were introduced by asymmetric epoxidation of **66** and subsequent treatment with lithium dimethylcyanocuprate in THF/ether which leads to diol **67**. After monoprotection of the secondary alcohol (three steps), further elaboration of the resulting monoprotected alcohol to diene **69** was achieved through oxidation of the primary alcohol to the corresponding aldehyde **68** which was treated with α-TMS-allylbromide **G** [61] and chromous chloride [62] and then with NaH to give diene **69** (80% overall yield). After deprotection of the acetal (Dibal-H), the iodide was prepared and converted to the trialkyl boranate **70** by lithiation and subsequent addition of *B*-methoxy-9-BBN (Scheme 14).

Scheme 14



A Suzuki-coupling reaction between **70** and vinyl iodide **63** in the presence of $PdCl_2$(dppf) [63] afforded the coupling product **71**, which represents the discodermolide skeleton. The final steps of the synthesis were the selective cleavage of the PMP acetal of **71**, oxidation (Dess-Martin and $NaClO_2$) [56,64] to the acid which was esterified ($TMSCHN_2$). After selective deprotection of the TES in **72**, (TsOH, MeOH, 0 °C), carbamoylation of the resulting hydroxy group at C19, deprotection of the PMB ether and prolonged exposure to 4N HCl, implying the cleavage of the remaining protecting groups and lactonization, (+)-discodermolide was obtained (Scheme 15).

Scheme 15




This synthesis illustrates the potential of chiral allenylmetal reagents for the synthesis of this polyketide natural product.

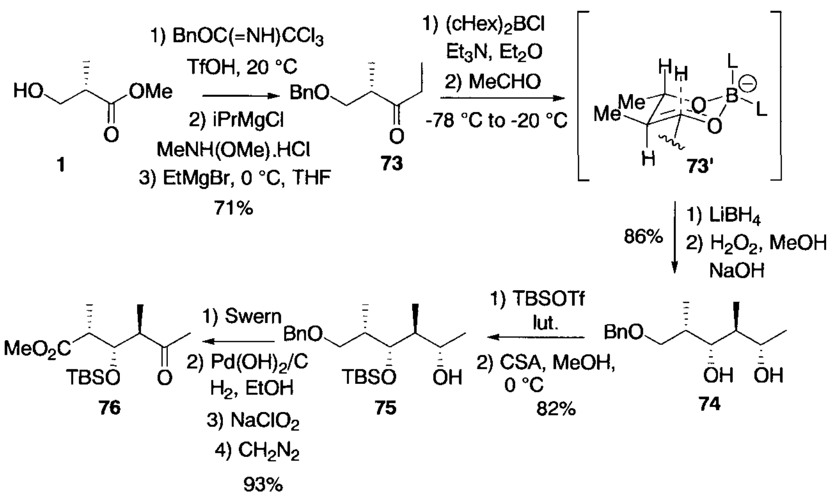
*Paterson's Synthesis*

Paterson's synthetic strategy is based on the coupling of three fragments of similar stereochemical and functional group complexity: the C1-C6, C9-C16 and C17-C24 subunits. The absolute stereochemistry of these fragments was established *via* substrate-controlled, boron-mediated aldol reactions of chiral ethylketones.

The synthesis of the C1-C6 subunit started with the α-chiral ethylketone **73** [65], prepared from the commercially available methyl (*S*)-2-methyl-3-hydroxypropionate **1** in three steps. Enolization of **73** by

using $(c\text{-Hex})_2BCl/Et_3N$ led to the selective generation of the (*E*)-boron enolate which underwent addition to acetaldehyde through a highly ordered chairlike transition state [65]. This was followed by the *in situ* reduction of intermediate boron aldolate **73'** with $LiBH_4$, via axial hydride delivery to provide after oxidative work-up, the 1,3-*syn* diol **74** (ds>97%) [66, 67]. After protection and selective deprotection, the monoprotected obtained diol **75** was oxidized to the corresponding ketoaldehyde (Swern). After debenzylation and further oxidation with sodium chlorite, the carboxylic acid was isolated and esterified with diazomethane to produce**76** (Scheme 16).

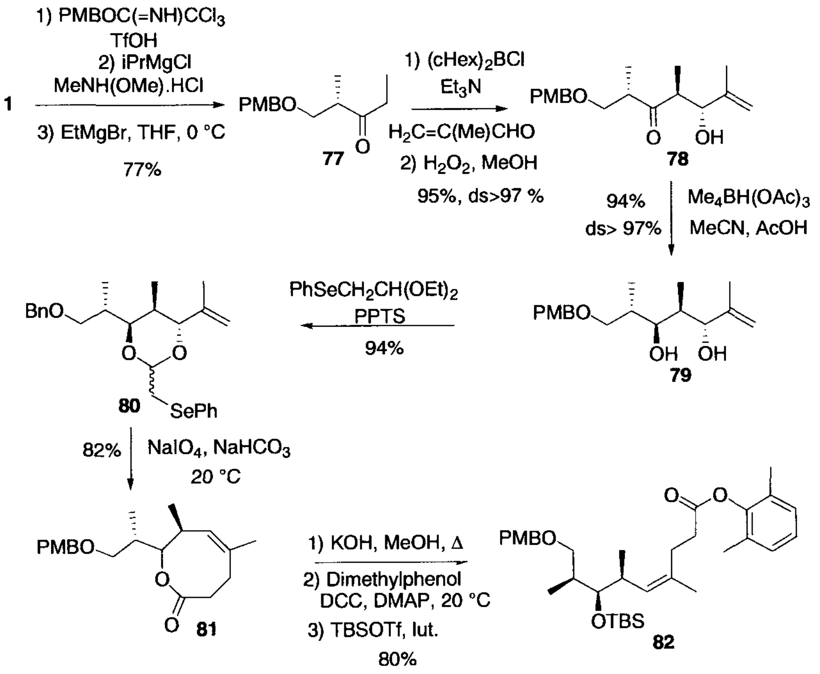
Scheme 16




The second fragment to be synthesized is the C9-C14 subunit, which contains the C13-C14 trisubstituted (*Z*)-olefin. The synthesis of this fragment started from the PMB protected ketone (*S*)-**77** obtained in three steps from the commercially available methyl (*S*)-2-methyl-3-hydroxypropionate **1**. The (*E*)-enolization of ketone (*S*)-**77** by using $(cHex)_2BCl/Et_3N$ and addition of methacrolein gave, after oxidative work-up, the expected aldol product **78** (ds>97%). The 1,3-*anti* reduction of the β-hydroxyketone by using $Me_4NBH(OAc)_3$ in MeCN and AcOH provided the 1,3-diol **79** (ds>97%). The introduction of the (*Z*)-double bond was achieved *via* the 2-phenylselenoacetaldehyde diethyl acetal **80** [68], which was oxidized ($NaIO_4$) and the crude product was directly subjected to the conditions of Claisen rearrangement to produce the

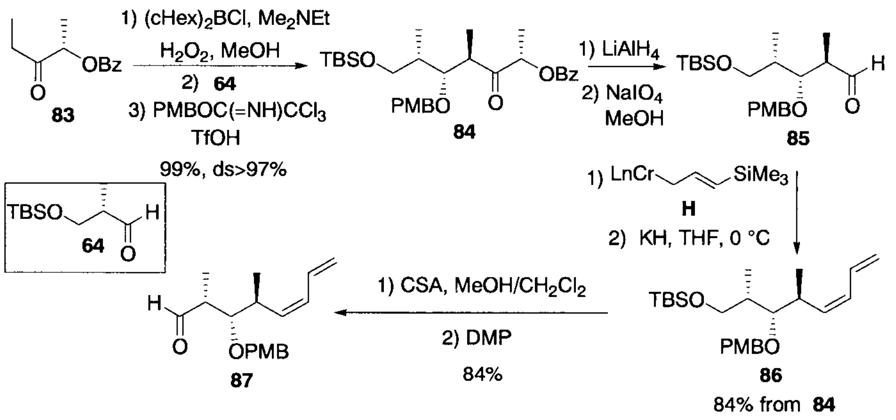
desired eight-membered lactone **81** (82% yield) with the C13-C14 (*Z*)-trisubstituted olefin. The conversion of lactone **81** to the arylester **82** in a three-step sequence involved a direct opening of the lactone **81**, the esterification with 2,6-dimethylphenol and a TBS protection of the secondary alcohol (Scheme 17).

Scheme 17




The last fragment, the C17-C24 fragment was synthesized from ethylketone (*S*)-**83** [69] prepared in three steps from the commercially ethyl (*S*)-lactate. Enolization of ketone (*S*)-**83** by using $(cHex)_2BCl/Me_2NEt$ generates the (*E*)-boron enolate which was added to aldehyde **64** to provide, after oxidative work-up, the *anti*-aldol product (ds>97%). After protection and reduction, the obtained diol was oxidatively cleaved to produce aldehyde **85**. A Nozaki-Hiyama reaction between aldehyde **85** and allylchromium reagent **H** [70] led to an *anti* β-hydroxysilane which was directly subjected to a Peterson-type *syn*-elimination (KH, THF) to provide the desired (*Z*)-diene **86** [71]. A deprotection and an oxidation (DMP) [57] led to the C17-C24 unit **87** (Scheme 18).

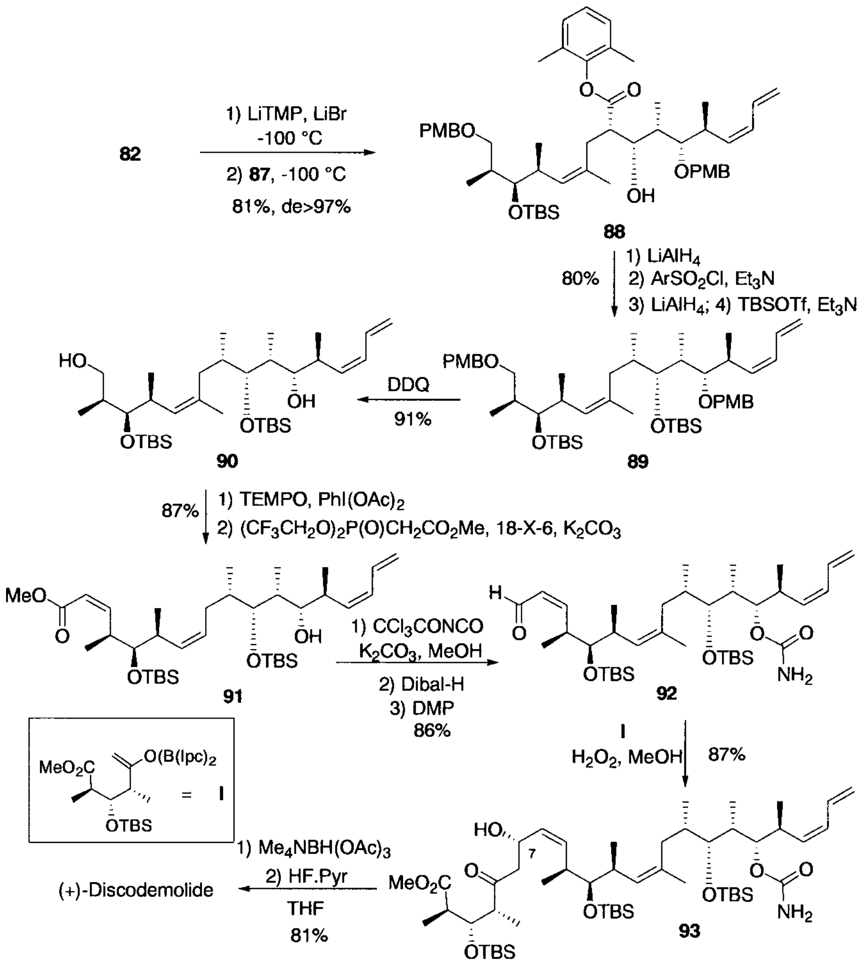
Scheme 18




The stereocontrolled union of the three components was achieved by using efficient aldol-based protocols. At first, the C16-C17 bond connection was realized by using a stereoselective, lithium-mediated aldol reaction of the C9-C16 fragment **82** with the C17-C24 aldehyde **87** employing LiTMP/LiBr [72] at -100 °C to generate the (*E*)-lithium enolate of **82**. The desired *anti*-aldol adduct **88** was obtained with high Felkin-Anh selectivity (ds>97%) (Scheme 19). After reduction of the aldol product **88**, the 1,3-diol was selectively desoxygenated by reduction of a monosulfate intermediate to produce **89** which after protection of the hydroxy group at C17 and deprotection of the hydroxy group at C9 and C19 led to the C9-C24 fragment which was then transformed to the C7-C24 fragment **90**. The introduction of the C8-C9 (*Z*)-olefin required the oxidation of the hydroxy group at C17. Diol **90** was chemoselectively converted to the aldehyde and the (*Z*)-olefin was introduced under mild Horner-Wittig-Emmons conditions [23], to provide the desired (*Z*)-enoate **91** (87% overall yield for the two steps). The C7-C24 aldehyde **92** was completed in a further three-step sequence, introduction of the carbamate moiety at C19 [54], chemoselective reduction of the unsaturated ester with Dibal-H at -78 °C and oxidation of the obtained allylic alcohol (DMP) furnished the desired aldehyde **92**. A successful union of boron enolate **I** with aldehyde **92** led to aldol products in 87% yield in a ratio of 84/16. The desired (7*S*) aldol product **93** was separable by chromatography from the minor (7*R*) epimer. The completion of the synthesis was achieved by an 1,3-*anti* reduction of the β-hydroxyketone [$(Me_4NBH(OAc)_3$], the final deprotection and γ-lactonization takes place

with HF.Pyr in THF to produce (+)-discodermolide (Scheme 19).

Scheme 19



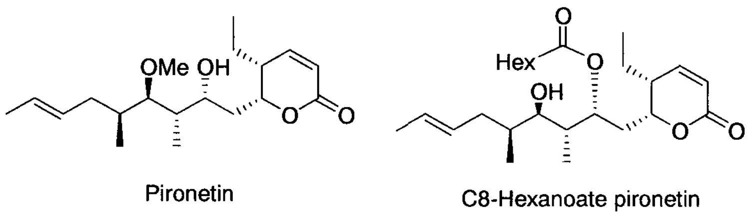

## Pironetin

***Isolation, Structure, Elucidation, Biosynthesis and Biological Activity.***

Pironetin was originally isolated from the culture broth of *Streptomyces sp* as a plant growth regulator which induced shortening of the plant height of rice [73, 74]. In 1996, it was also reported that PA-48153 C, which was identical to pironetin and showed potent immunosuppressive

effects on the blastogenesis of both T and B cells [75]. Slight modifications of the 2-pyran ring (*e.g.* 3,4-saturated, 3,4-epoxy, 3,4-cyclopropyl, 2-methoxy, 5-demethyl, or ring opened derivatives) or the side chain (*e.g.* 8-β-hydroxy-, 8-oxo-, 13,14-epoxy-, 13,14-*cis*-vinylene-, 14-methoxycarbonyl-15-nor-, or 13-oxo- derivatives) diminished the inhibitory activity on mitogen responses. Exceptionally, 5-β-methoxy, 14-methyl, 14,14-dibromo-15-nor, 8-*O*-acyl, 8-*O*-carbamoyl derivatives retained the potent inhibitory activity on the responses of both T and B cells to mitogen. Among these derivatives, the C-8α-alcohol had weakened cytotoxicity to EL4 cells, and the C8-hexanoate showed potent suppressive effects on mitogen responses with less cytotoxicity to EL4 cells and this compound was selected for *in vivo* evaluation [75] (Fig 2).

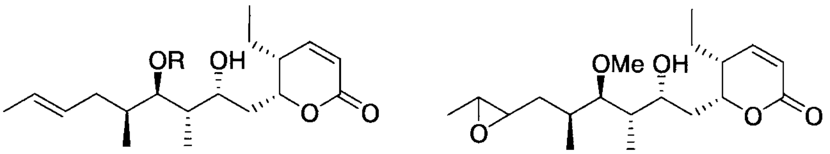
Figure 2




More recently in 1998, Osada [76] showed that pironetin inhibited the cell cycle progression in the M-phase and showed antitumor activity against transplanted murine tumor cell lines P 388 leukemia in mice. A major limitation of pironetin as a therapeutic agent was its high cytotoxicity. However, certain structurally modified derivatives of pironetin have recently shown promise [77]. Moreover, these relatively simple structures exhibit potency comparable to that shown by cyclosporin. Another recent report in the patent literature suggests efficacy of certain formulations of such derivatives in assays against uterine and ovarian tumors, in addition to activity as inhibitors of tubulin polymerization and thus the cell cycle [75]. The effect of pironetin and its derivatives on the distribution of the cellular DNA contents in exponentially growing 3Y1 cells was examined for colcemid, pironetin, NK 109589 and epoxypironetin (Fig. 3). Colcemid, a mitotic arrester, induced the M-phase arrest as represented by the cell population containing 4C DNA. Pironetin and its demethyl derivative, NK 109 58P, also arrested the cell cycle progression at G2/M in the concentrations

above 20 ng/mL. The cells were also arrested at the G2/M phase by epoxypironetin but only in concentrations higher than that of pironetin and NK 109 58 P [76] (Fig. 3).

Figure 3




R = $CH_3$, Pironetin
R = H, NK 10958P

Epoxypyronetin

Table 1: Distribution of DNA contents in 3Y1 cells treated with various drugs

| | Concentration (ng/mL) | DNA content | | |
|---|---|---|---|---|
| | | 2C | 2-4C | 4C |
| Control | 0 | 61.0 | 16.5 | 16.5 |
| Colcemid | 100 | 4.4 | 4.9 | 62.8 |
| Pironetin | 20 | 11.6 | 10.0 | 64.6 |
| | 50 | 7.2 | 10.6 | 68.1 |
| | 100 | 6.4 | 10.3 | 70.5 |
| NK 10958P | 20 | 33.1 | 10.6 | 44.7 |
| | 50 | 12.9 | 10.4 | 65.4 |
| | 100 | 9.5 | 9.9 | 67.1 |
| Epoxypironetin | 200 | 58.6 | 15.9 | 16.3 |
| | 500 | 7.9 | 10.0 | 73.2 |
| | 1000 | 4.5 | 7.8 | 77.1 |

Exponentially growing 3Y1 cells were treated with various drugs for 24 h and the distribution of DNA content and relative cell number (cell number at 24 h per initial cell number at 0 h x 100) were determined.

There are many cancer therapeutic agents, which inhibit the metaphase progression. So, the *in vivo* antitumor activity of pironetin in

mice injected with the murine leukemic cell line P 388 was tested (Table 2). The antitumor activity of pironetin was very modest compared with that of synthetic alkylating agents. However, the results obtained were encouraging because pironetin arrested the cell cycle at the M-phase where cells are most sensitive to DNA attacking reagents, and therefore, a combination therapy could be effective [76].

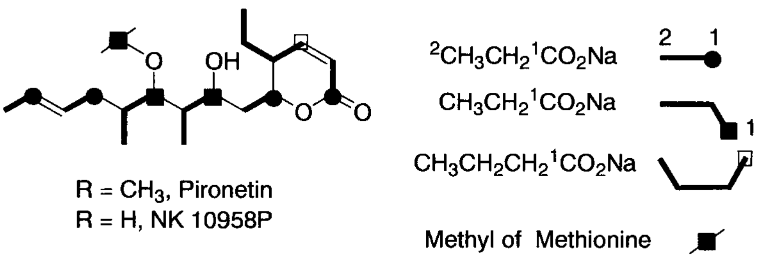
Table 2: Antitumour activity of pironetin againt P388

| Compound | Dose (mg/kg) | Body weight change (g) | Mean survival time (days) | T/C (%) |
|---|---|---|---|---|
| Control | 0.00 | 5.3 | 11.2 ± 0.84 | 100 |
| Pironetin | 0.78 | 3.7 | 11.2 ± 0.58 | 104 |
| | 1.56 | 0.5 | 11.7 ± 0.58 | 104 |
| | 3.13 | -0.07 | 13.3 ± 1.15 | 119 |
| | 6.25 | -2.6 | 14.3 ± 0.58 | 128 |

Approximately $1x10^5$ P388 cells of murine leukemia cells were injected intraperitoneally into $CDF_1$-SLC mice (10 weeks, female on day 0). Pironetin was administrated intraperitoeally daily for days. Each mouse was weighed on day 1 and 10, and mortality was monitored.

The structure of pironetin was determined to be (5*R*,6*R*)-5-ethyl-5,6-dihydro-6[(*E*)-(2*R*,3*S*,4*R*,5*S*)-2-hydroxy-4-methoxy-3,5-dimethyl-7-nonenyl]-2H-pyran-2-one by FAB-MS, $^1H$ and $^{13}C$ NMR, COSY, COLOC, DEPT, IR, X-ray crystallographic analyses and adapted Mosher's method to determined the absolute configuration [74]. The biosynthesis of pironetin appeared to involve assembly of acetate, propionate and butyrate precursors. Four fermentation experiments were carried out in order to determine the biosynthetic origin of the carbon skeleton and oxygen atoms of pironetin. [1-$^{13}$C]Acetate, [2-$^{13}$C]acetate, [1,2-$^{13}$C]acetate, [1-$^{13}$C]propionate, [1-$^{13}$C]butyrate and L-[*methyl*-$^{13}$C]methionine were led separately to *Streptomyces sp.* culture and pironetin was isolated and purified. Analysis of pironetin by $^{13}C$ NMR showed that pironetin was derived from four acetate units, two propionate units, one butyrate unit and one methyl unit of methionine [78] (Fig. 4).

Figure 4



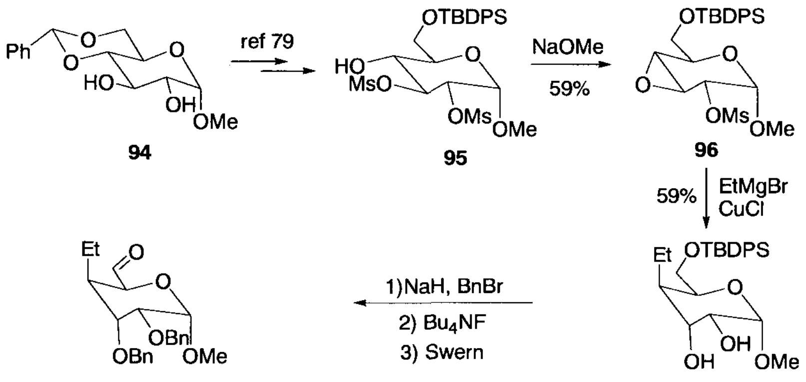

***Synthetic Routes to (-)-Pironetin***

Not only the interesting activities but also the unique and simple structure make pironetin and its derivatives attractive synthetic targets and seven total synthesis of pironetin have been reported.

*Kawada's Synthesis [77].*

Kawada and et reported the first convergent synthesis of (-)-pironetin, which utilized a carbohydrate approach to the 2-pyranone ring system. (-)-Pironetin was synthetized starting from (+)-methyl 4,6-*O*-benzylene-α-D-glucopyranoside **94** and (*S*)-(+)methyl 3-hydroxy-2-methylpropionate **1**. The key steps for the transformation of **94** to **98** relied upon *trans*-diaxal ring opening of an epoxide. The synthesis of the desired epoxide **96** was achieved via the dimesylate **95** which was obtained in three steps from **94** by a known procedure [79]. Treatment of the dimesylate **95** with sodium methoxide gave the required epoxide **96** and its ring opening with ethylmagnesium chloride in the presence of CuCl [80] led to the introduction of the expected axial ethyl group into the C5 position (59% yield). In addition, an excess of Grignard reagent caused the subsequent demesylation and provided diol **97**. After protection-deprotection sequence and a Swern oxidation, the aldehyde **98**, precursor of the pyranone ring, was isolated. This sequence allows the control of the C5 and C6 stereogenic centers (Scheme 20).

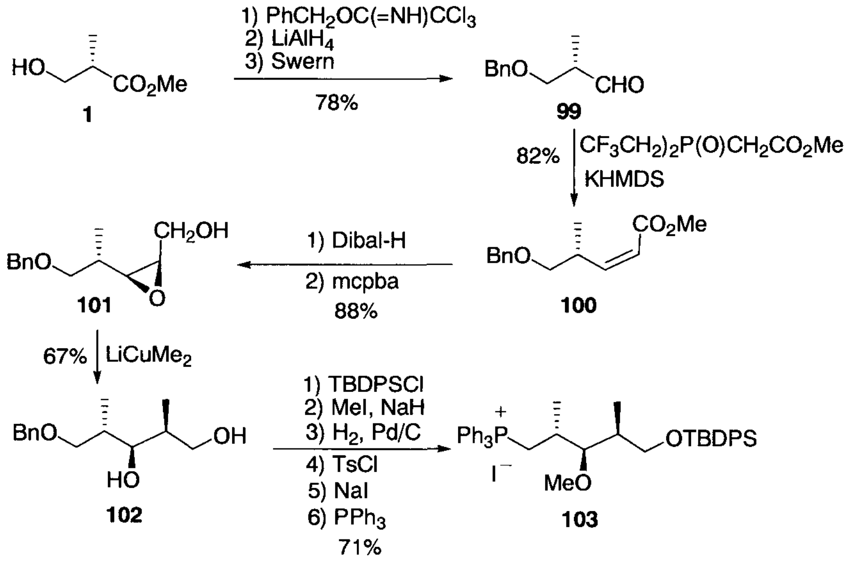
Scheme 20




94 95 96 97 98

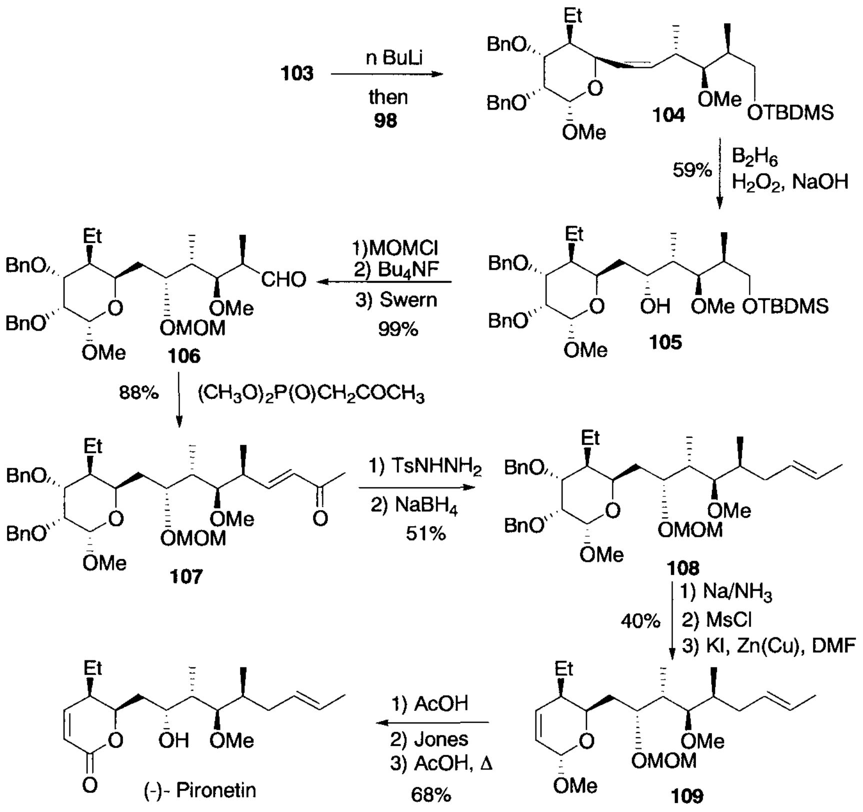
The construction of the alkenyl side chain and the control of the C9, C10 and C11 stereogenic centers was achieved from (*S*)-(+)-methyl 3-hydroxy-2-methylpropionate **1**. (Scheme 21) This compound was transformed to aldehyde **99** in three steps. Bis(2,2,2)trifluoroethyl)[(methoxycabonyl)methyl]-phosphonate [23] was employed for the selective formation of the *cis*-α,β-unsaturated ester **100**. From this Z-unsaturated ester **100**, the three consecutive asymmetric units were constructed *via* epoxide **101** (*m*-CPBA), which was selectively opened by lithium dimethylcuprate to produce **102**. After deprotection-protection, the alcohol **102** was converted to the phosphonium iodide **103** *via* a tosylate intermediate(Scheme 21).

Scheme 21




The two optically active segments **98** and **103** were condensed (Scheme 22). The deprotonation of the phosphonium salt **103** with *n*-BuLi gave the phosphorus ylide, and the coupling reaction with aldehyde **98** proceeded smoothly to stereoselectively afford (*Z*)-olefin **104**. The control of the stereogenic center at C8 was achieved by hydroboration of **104** ($B_2H_6$, $H_2O_2$, NaOH), which was found to be regio- and stereoselective (59% yield). After a three-step sequence, protection-deprotection-oxidation, the aldehyde **106** was transformed to the *trans*-α,β-unsaturated ketone **107** by using a Horner-Emmons reaction [dimethyl(2-oxopropyl)-phosphonate], ketone **107** was converted to the olefinic compound **108** after reductive deoxygenation of a carbonyl tosylhydrazone with sodium borohydride in acetic acid [81], which implies the stereoselective migration of the double bond to the desired position. To introduce the double bond at C3, **108** was converted to the bis-mesylate (2 steps) which after removal of the vicinal sulfonyloxy groups (KI, Zn-Cu, DMF) led to the protected γ-lactol **109** [82]. Selective hydrolysis of the lactol ($CH_3CO_2H$/ $H_2O$, 40 °C) oxidation (Jones reagent) and deprotection of the MOM group ($CH_3CO_2H$, reflux) provided (-)-pironetin (Scheme 22).

Scheme 22




Various (-)-pironetin-related compounds were prepared using this synthetic route or by partial synthesis from natural (-)-pironetin, in particular by modification of the C8 alcohol.

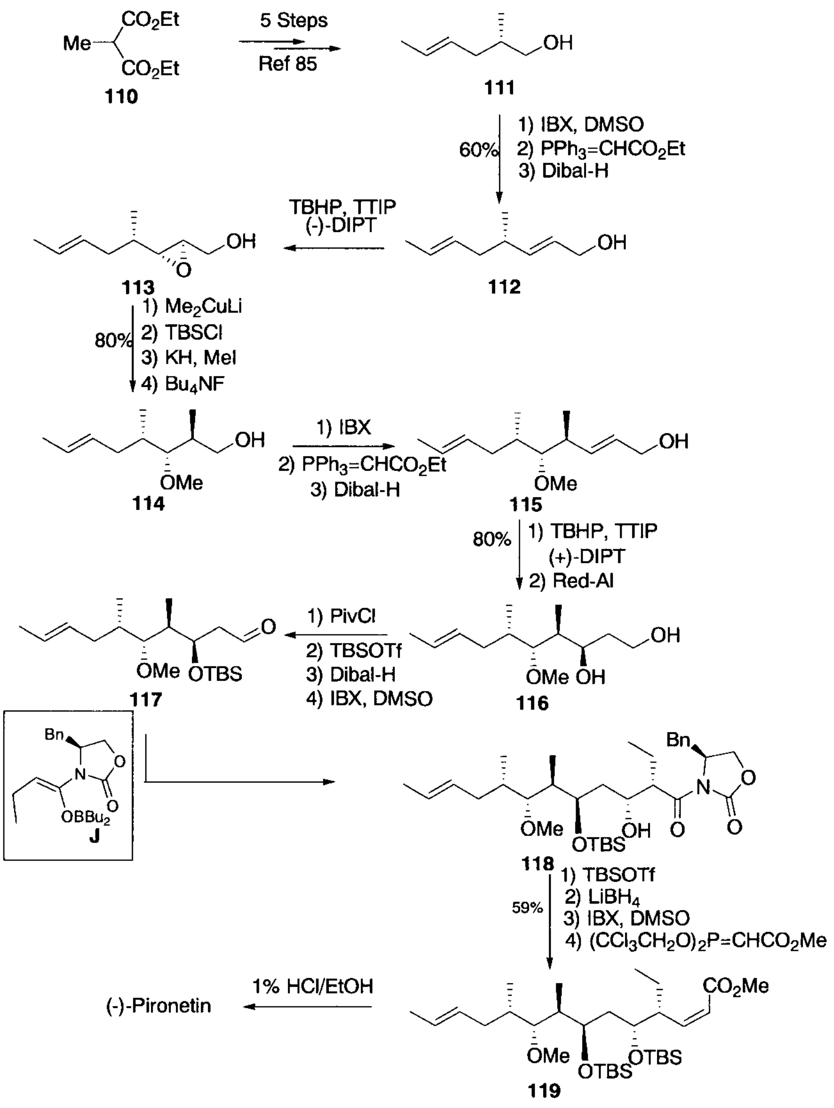
*Gurjar's Syntheses [83, 84].*

Gurjar et al. have developed two strategies, which could be adapted for the preparation of pironetin and of its analogues. The key steps in Gurjar's syntheses, to control the stereogenic centers, are the regioselective opening of epoxides obtained by a Sharpless epoxidation and an Evans aldol condensation.

The first route [83] began with dimethylmalonate that was

transformed to (2*S*,4*E*)-2-methylhexen-1-ol **111** on large scale in a five-step sequence [85]. The alcohol **111** was successively oxidized (IBX, DMSO), olefinated ($PPh_3$=CH-$CO_2$Et) and reduced (Dibal-H) to afford the allylic alcohol **112** which was submitted to a Sharpless asymmetric epoxidation [86]. The resulting epoxyalcohol **113** was subsequently treated with $Me_2CuLi$ to give after a protection-deprotection sequence, alcohol **114**. The ring opening of epoxide **113** by $Me_2CuLi$ allowed the control of the C12, C13 and C14 stereogenic centers. Alcohol **114** was then transformed to the allylic alcohol **115** in three steps (Scheme 23). After a Sharpless epoxidation [(+)-DIPT] and a regioselective reduction of the obtained epoxide (Red-Al), the obtained alcohol was transformed to aldehyde **117** (four steps). The control of the stereogenic centers at C4 and C5 was achieved by using an Evans aldol condensation between aldehyde **117** and the dibutyl enolate **J** [87] which produces **118** with high diastereoselectivity. Protection of the free alcohol, reduction ($LiBH_4$) and oxidation (IBX, DMSO) led to an aldehyde which by a *Z*-olefination with a modified Horner-Wadsworth-Emmons reaction [$(CCl_3CH_2O)_2$P-$CO_2$Me] [88] gave **119** as the sole product. Finally (-)-pironetin was obtained from **119** by treatment with 1% HCl in EtOH (Scheme 23).

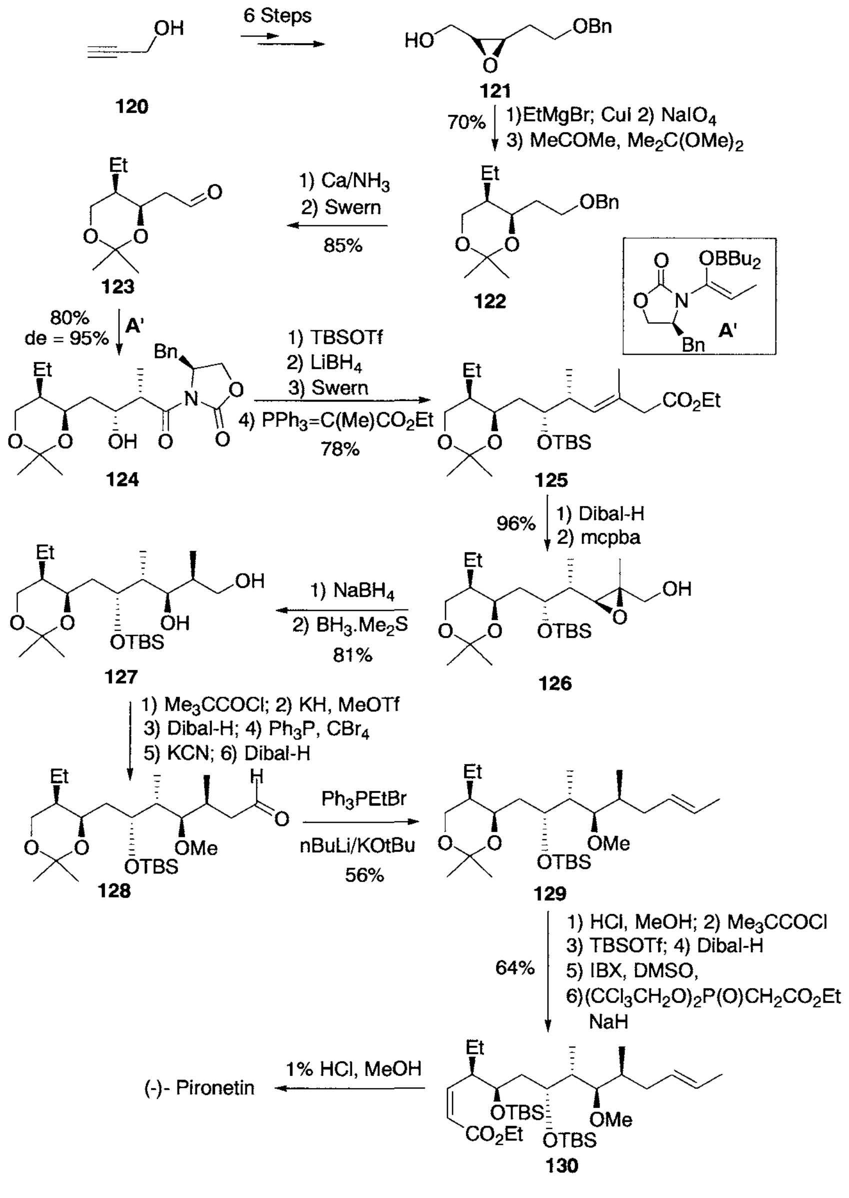
Scheme 23




For their second approach [84], Gurjar et al. have explored an Evans asymmetric aldol reaction, and a regiospecific ring opening of epoxides. They started their synthesis from (2*S*,3*R*)-epoxy-alcohol **121**, which was prepared from propargyl alcohol **120** in six high yielding steps

[89]. Subsequent Grignard reaction on **121** using EtMgBr in the presence of CuI followed by periodate oxydation and a protection step provided **122** (70%) which was transformed to aldehyde **123**. An Evans asymmetric aldol reaction employing the dibutyl enolate **A'** at -78 °C gave **124**, with a high diastereomeric purity (de = 95%). The transformation of **124** to the allylic alcohol **125** was achieved in four steps and a Wittig reaction ($Ph_3P{=}(Me)CO_2Et$) allowed the control of the *E* olefin. Reduction of **125** and treatment of the obtained alcohol with *m*-CPBA cleanly produced epoxide **126** (96% yield) as a single product, which was transformed selectively to alcohol **127** ($NaBH_4$, then $BH_3.Me_2S$, 81% yield) [90]. At this stage, introduction of the *E*-propenyl group was considered. The transformation of **127** to aldehyde **128** was a straight forward exercise (six steps). Aldehyde **128** was transformed to olefin **129** by using the ylide, $Ph_3P{=}CHCH_3$, under Schlosser conditions [91] to give the (*E*)-product (56% yield). Compound **129** was then converted to the unsaturated ester **130** by a deprotection-protection sequence followed by oxidation of the primary alcohol and modified Wittig conditions by using $(CCl_3CH_2O)_2P(O){=}CHCO_2Et$. As previously, **130** was transformed to (-)-pironetin by using the same conditions (Scheme 24).

## Scheme 24



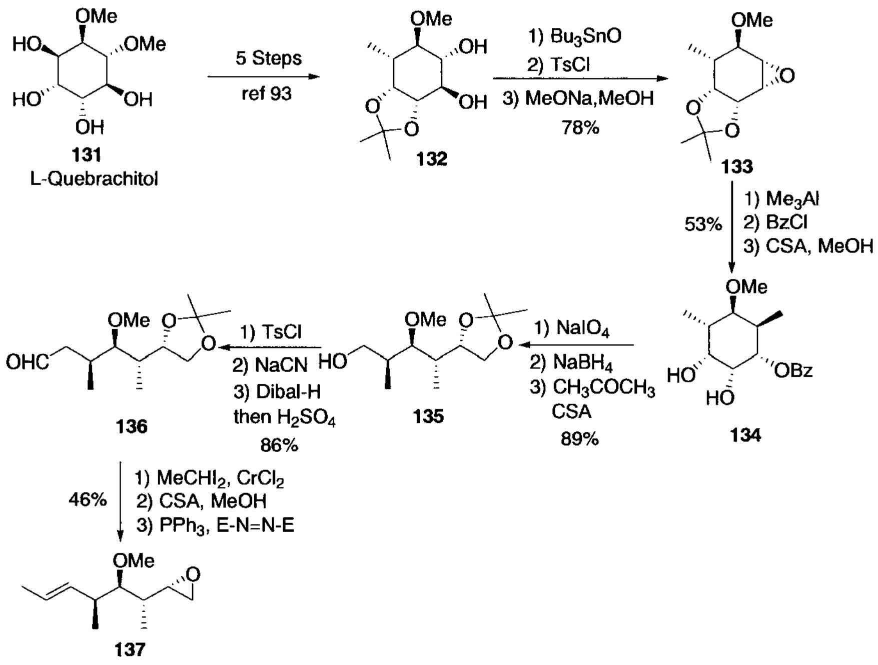
6 Steps
120
121
70% 1)EtMgBr; CuI 2) NaIO4 3) MeCOMe, Me2C(OMe)2
122
1) Ca/NH3 2) Swern 85%
123
A'
80% de = 95%
124
1) TBSOTf 2) LiBH4 3) Swern 4) PPh3=C(Me)CO2Et 78%
125
96% 1) Dibal-H 2) mcpba
126
1) NaBH4 2) BH3.Me2S 81%
127
1) Me3CCOCl; 2) KH, MeOTf 3) Dibal-H; 4) Ph3P, CBr4 5) KCN; 6) Dibal-H
128
Ph3PEtBr nBuLi/KOtBu 56%
129
64% 1) HCl, MeOH; 2) Me3CCOCl 3) TBSOTf; 4) Dibal-H 5) IBX, DMSO, 6)(CCl3CH2O)2P(O)CH2CO2Et NaH
130
1% HCl, MeOH
(-)- Pironetin

*Chida's Synthesis [92]*

In 1997, Chida et al. presented a convergent synthesis of (-)-pironetin starting from L-quebrachitol **131** and L-malic acid. The acyclic portion of (-)-pironetin possessing four contiguous chiral centers were constructed stereoselectively from L-quebrachitol and the 2-pyrane moiety was prepared from L-malic acid. In this synthesis, the nucleophilic addition of a dithiane to epoxide is a strategic key step.

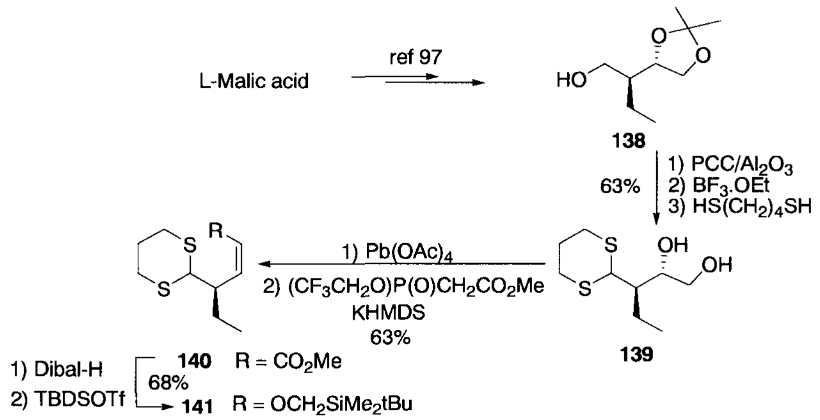
The known 1D-(1,2,3,5/4,6)-1,2-*O*-iso-propylidene-3-methyl-4-*O*-methylcyclohexane-1,2,5,6-pentol **132** [93], prepared stereoselectively from L-quebrachitol in five steps, was chosen as the starting material for the preparation of the acyclic moiety of (-)-pironetin. Selective tosylation of **132** by using dibutyltin oxide [94] and tosyl chloride followed by a base treatment led to the α-epoxide **133**. The *trans*-diaxal opening of the epoxide ring with trimethylaluminium afforded the hexasubstituted cyclohexane which was transformed after a protection-deprotection sequence to **134**. The cyclohexane ring in **134** was cleaved by using a periodate oxidation and the resulting acyclic derivative was not isolated but reduced with $NaBH_4$ to afford a diol (90% yield) which was protected to produce **135**. This compound was then transformed to aldehyde **136** in three steps. After a Takai reaction ($E/Z$ = 11/1 for the obtained olefin) [95], a deprotection and a Mitsunobu reaction [96], aldehyde **136** was transformed to epoxide **137** which corresponds to the C6-C14 fragment of (-)-pironetin (Scheme 25).

Scheme 25



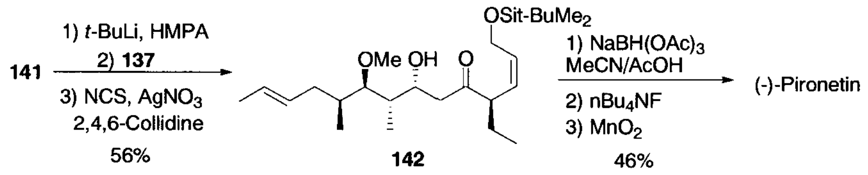

The synthesis of the precursor of the 2-pyranone portion of (-)-pironetin started from the known (2*S*,3*S*)-2-ethyl-3,4-isopropylidenedioxypropan-1-ol **138**, obtained from L-malic acid in six steps [97]. Oxidation of **138** with PCC on alumina [98], followed by treatment with propane-1,3-dithiol ($BF_3.OEt_2$) gave diol **139** (63% yield). Cleavage of the glycol and subsequent modified Wittig-Horner reaction [23] gave the desired *Z*-alkene **140** which was transformed to the protected alcohol **141** after reduction with Dibal-H and protection of the resulting function (Scheme 26).

Scheme 26



Deprotonation of **141** with *t*-Buli in the presence of HMPA [99] followed by the addition of epoxide **137** (Scheme 25) afforded the coupling product which after deprotection of the thioketal (NCS, $AgNO_3$, 2,4,6-collidine) [100] produced ketone **142**. A selective reduction of ketone **142** to the *anti*-diol ($NaBH(OAc)_3$, MeCN/AcOH) [51, 101], removal of the protecting *O*-silyl protecting group and selective $MnO_2$ oxidation furnish (-)-pironetin (Scheme 27).

Scheme 27

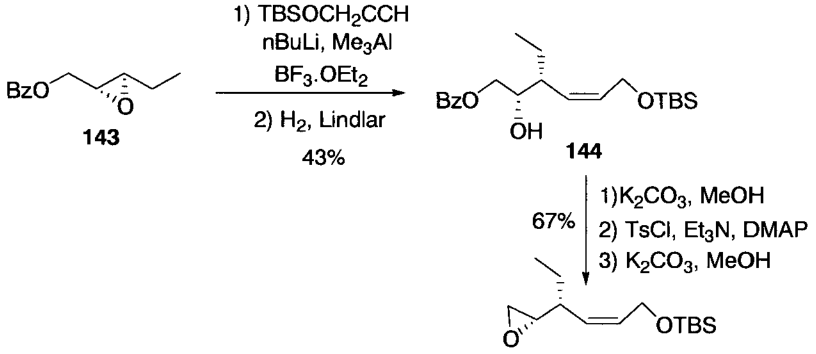


*Kitahara's Synthesis [102,103]*

In 1998 [102], Kitahara et al. reported also a convergent total synthesis of (-)-pironetin by using three chiral buiding blocks: the (1*S*,5*S*,6*R*)-5-hydroxy-bicyclo[4.1.0]heptan-2-one, a dithiane and an epoxide. As in the previous synthesis, the nucleophilic addition of the dithiane to the epoxide

played a prominent strategic role. The preparation of the C1-C6 was achieved from the known epoxide **143** [104]. Regioselective epoxide opening of **143** using acetylenic alanate followed by hydrogenation, over Lindlar's catalyst, gives *Z*-homoallylalcohol **144**. Methanolysis of the benzoate, tosylation of the primary hydroxy group, followed by epoxide formation under basic conditions afforded the C1-C6 unit **145** (overall yield from **143**, 28%) (Scheme 28).

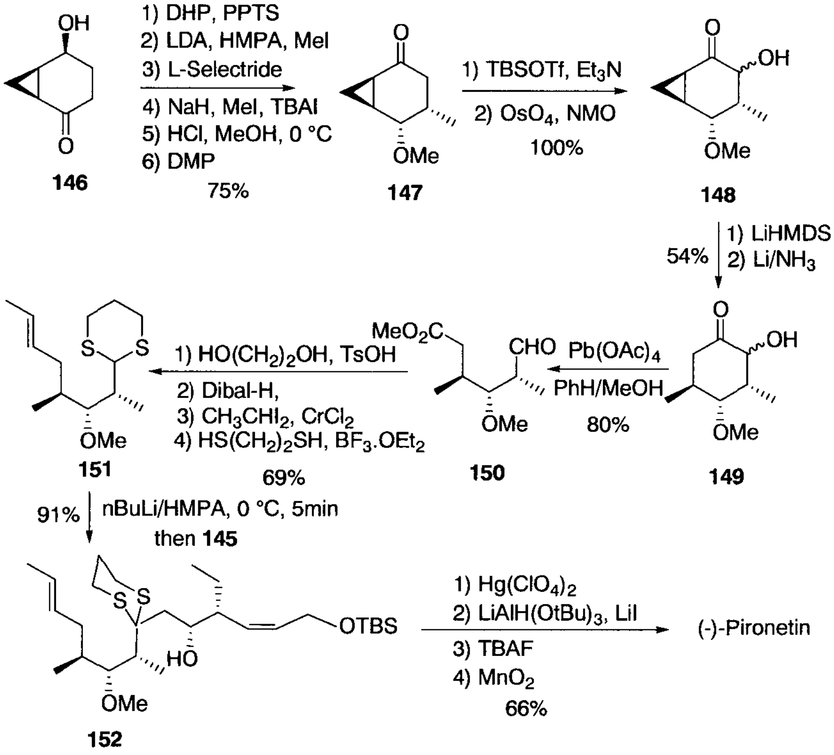
Scheme 28




The C7-C14 unit was prepared from the (1*S*,5*S*,6*R*)-5-hydroxybicyclo[4.1.0]heptan-2-one **146** [105], which was transformed in six steps to the bicyclic[4.1.0]heptanone **147**. Oxidation of the silyl enol ether of **147** with osmium tetroxide gives the α-hydroxyketone **148** in quantitative yield as an inseparable mixture (α:β = 2:3). Reduction of the cyclopropyl moiety *via* lithium alkoxide under Birch conditions, led to **149** as a mixture of isomers. The ketol moiety of **149** was oxidatively cleaved with lead tetraacetate in benzene-methanol to give the single aldehyde ester **150**, which was transformed to dithiane **151** after a protection-reduction sequence, a Takai reaction [95] to introduce the *E*-olefin, and a thioacetalisation of the ethylene acetal (Scheme 29). The coupling reaction of the two units **145** and **151** successfully gave **152**, which possesses the whole carbon skeleton of (-)-pironetin. The key feature of this reaction was the formation of the anion of **151** by treatment of the thiocetal with *n*-Buli at 0 °C for five minutes. The obtained coupling product **152** was transformed to pironetin after dethioketalisation ($HgClO_4$) selective reduction [106] of the obtained ketone to the *anti*-diol ($LiAlH(OtBu)_3$, LiI, -78 °C to 0 °C), desilylation

and selective oxidation ($MnO_2$) (Scheme 29).

Scheme 29

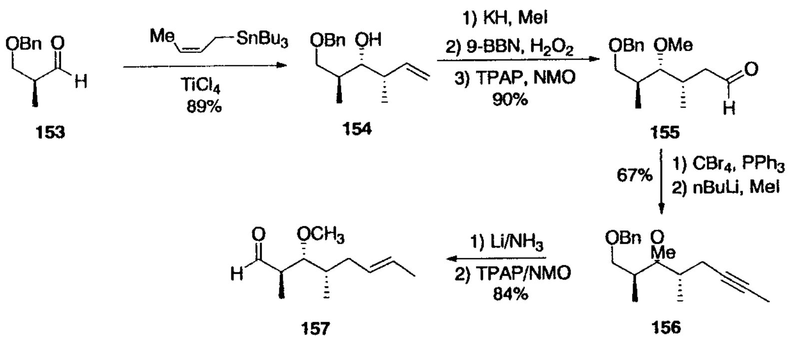



*Keck's Synthesis [107]*

Keck et al. have used the lactone annulation procedure developed in their laboratory to install the lactone moiety by reaction of the lithium enolate of methylacetate with a β-acetoxy aldehyde [108]. The other key steps include a diastereoselective Lewis acid mediated (*Z*)-crotylstannane aldehyde addition, a highly selective Lewis acid promoted Mukaiyama aldol reaction and an *anti*-selective $SmI_2$ reduction of a β-hydroxyketone. The preparation of the C8-C15 fragment of (-)-pironetin started with the chelation-controlled addition of (*Z*)-crotyl tri-*n*-butylstannane to the β-benzyloxy-aldehyde **153** in the presence of $TiCl_4$ [109] to give the

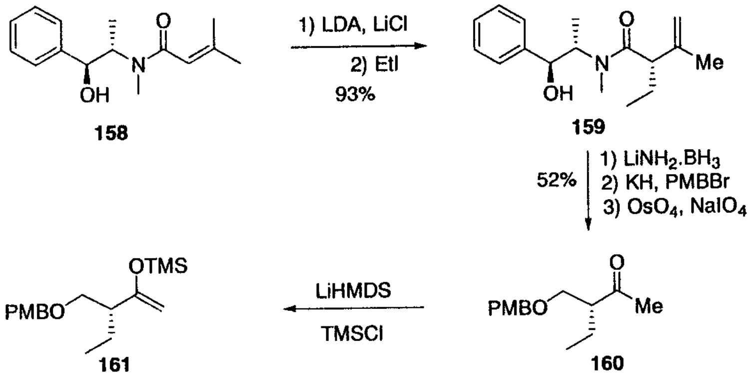
desired *anti,syn*-homoallylic alcohol **154** (89%) which was converted to aldehyde **155** after protection of the free hydroxy group and hydroboration-oxidation of the primary alcohol. After transformation of **155** to alkyne **156** by using a Corey-Fuchs' protocol [110], reduction (Li/$NH_3$) and oxidation (TPAP, NMO) [111], the desired *E*-unsaturated aldehyde **157** was isolated and used immediatly in the subsequent Mukaiyama aldol reaction (Scheme 30).

Scheme 30



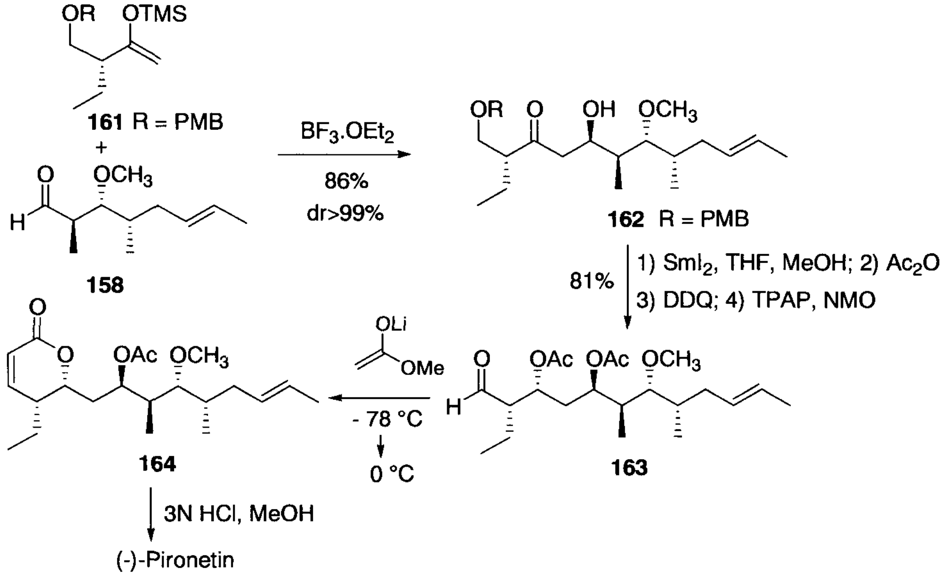

The preparation of the methyl ketone required for the aldol coupling reaction was accomplished by using the asymmetric alkylation of the unsaturated amide **158** according to a protocol developed by Myers [112]. Asymmetric alkylation of **158** with ethyl iodide gave **159** which was reduced to the primary alcohol ($LiNH_2$, $BH_3$) and protected as a PMB ether to produce, after oxidative cleavage of the olefin, the methyl ketone **160** which was converted to the trimethylsilyl enol ether **161** (LiHMDS, TMSCl) (Scheme 31).

Scheme 31



The Mukaiyama aldol condensation of **158** and **161** in the presence of $BF_3.OEt_2$ proceeded to yield **162** as a single diasteereomer (86%, dr > 99%). Reduction of ketone **162** by using $SmI_2$ [113] allowed the control of the C6 stereogenic center with a reasonable diastereoselectivity (dr = 4.6/1). After acetylation, deprotection and oxidation, the aldehyde **163** was properly functionalized to effect incorporation of the lactone ring on a single step [108]. Aldehyde **163** was then treated with the lithium enolate of methyl acetate to form the desired lactone **164**. Finally, removal of the acetate by acid-catalyzed methanolysis gave (-)-pironetin (Scheme 32).

Scheme 32




*Dias' Synthesis [114]*

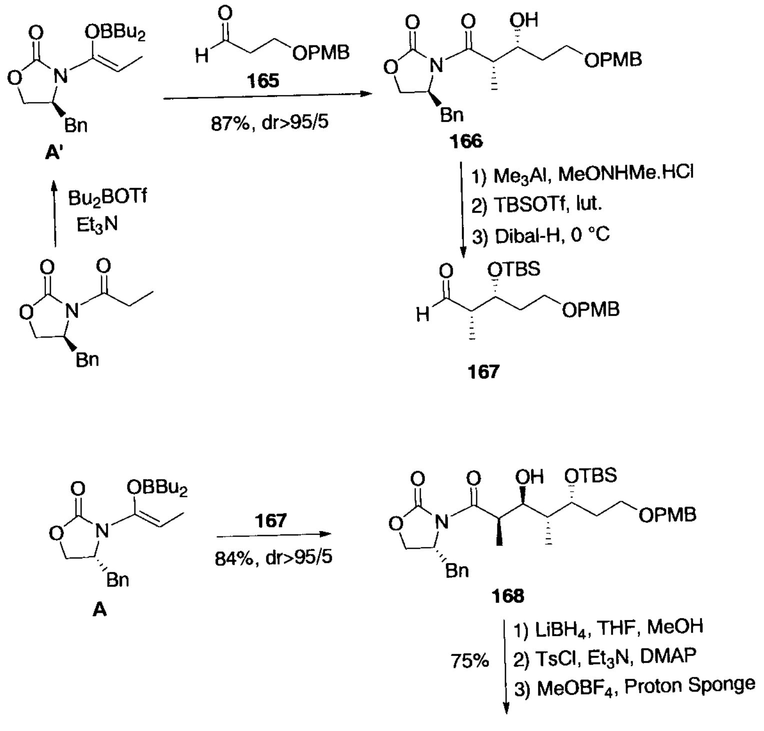
The Dias' approach involves the use of three very efficient Evans oxazolidinone-mediated *syn*-aldol condensations. The other key steps involve a coupling reaction between the lithium acetylide-ethylenediamine complex and a tosylate followed by a methylation and a selective reduction to establish the C12-C13 (*E*) double bond.

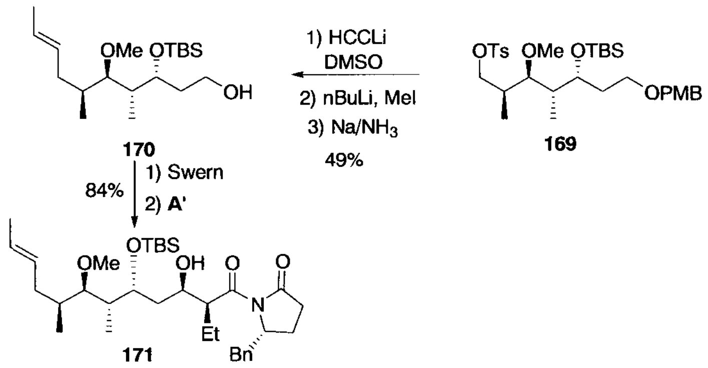
The synthesis of (-)pironetin began with the known (*S*)-acyloxazolidone. Asymmetric aldol addition of the boron enolate of **A'** with aldehyde **165** gave the *syn*-aldol adduct **166** with an excellent

diastereoselectivity (87%, dr>95/5). The adduct **166** was then transformed to aldehyde **167** in 3 steps and treated with the boron enolate **A** to give the corresponding aldol adduct **168**, which possesses the *syn,anti,syn* stereochemistry (84 %, dr = 95/5). After a three-step sequence, reduction-tosylation-methylation, the tosylated protected stereotetrad **169** was isolated (Scheme 33).

The synthesis of the fragment C3-C13 was achieved in five steps from **169**. Treatment of the tosylated stereotetrad **169** with 5 equivalents of lithium acetylide in DMSO led an acetylenic compound which was treated with *n*-Buli and methyl iodide, and then reduced by $Na/NH_3$ to produce the *E*-geometry of the C12-C13 double bond with concomitant removal of the PMB group at C5, giving the primary alcohol **170** (49% yield for the three-step sequence). Swern oxidation of **170** gave the corresponding aldehyde which was involved in an Evans-type asymmetric aldol reaction with the boron enolate **A'** to produce the adduct **171** (dr > 95/5, 90% yield). (Scheme 33).

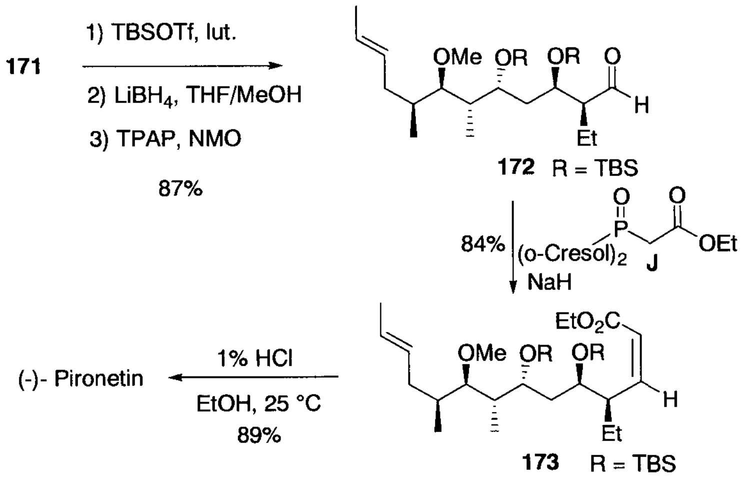
Scheme 33






The final steps of the synthesis are summarized in Scheme 34. After a sequence of protection-reduction-oxydation, **171** was transformed to aldehyde **172** which was directly submitted to a Horner-Wadsworth-Emmons homologation with the requisite stabilized reagent **K** under Ando'conditions [115] to give the corresponding α,β-unsaturated ester **173** (*Z*/*E* > 95/5) in 84% yield. Treatment of ester **173** with 1% HCl, EtOH led to (-)-pironetin after purification (89% yield).

Scheme 34




This synthesis required 18 steps from **A'** and the overall yield in (-)pironetin is 11 %. This synthesis is probably one of the shortest approaches of pironetin.

## REFERENCES

[1] Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Schulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912. Additions and corrections: *J. Org. Chem.* **1991**, *56*, 1346.

[2] Gunasekera, S. P.; Pomponi, S. A.; Longley, R. E. U.S. Patent No. US5840750, Nov 24, 1998.

[3] a) Hung, D. T.; Nerenberg, J. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 11054. b) Nerenberg, J. B.; Hung, D. T.; Somers, P. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 12621.

[4] Gunasekera, S. P.; Cranick, S.; Longley, R. E. *J. Nat. Prod.* **1989**, *52*, 757.

[5] a) Longley, R. E.; Caddigan, D.; Harmody, D.; Gunasekera, M.; Gunasekera, S. P.
*Transplantation* **1991**, *52*, 650. b) Longley, R. E.; Caddigan, D.; Harmody, D.; Gunasekera, M.; Gunasekera, S. P. *Ibid.* **1991**, *52*, 656. c) Longley, R. E.; Gunasekera, S. P.; Faherty, D.; McLane, J.; Dumont, F. *Ann. N. Y. Acad. Sci.* **1993**, *696*, 94.

[6] a) ter Haar, E.; Kowalski, R. J.; Hamel, E.; Lin, C. M.; Longley, R. E.; Gunasekera, S. P.; Rosenkranz, H. S.; Day, B. W. *Biochemistry* **1996**, *35*, 5, 243. b) Hung, D. T.; Chen, J.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 287.

[7] Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15.

[8] Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325.

[9] Long, B. H.; Carboni, J. M.; Wasserman, A. J.; Cornell, L. A.; Casazza, A. M.; Jensen, P. R.; Lindel, T.; Fenical, W.; Fairchild, C. R. *Cancer Res.* **1998**, *58*, 1111.

[10] Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653.

[11] Hung, D. T.; Chen, J.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 287.

[12] Kowalski, R. J.; Giannakakou, P.; Gunasekera, S. P.; Longley, R. E.; Day, B. W.; Hamel, E.
*Mol. Pharm.* **1997**, *52*, 613.

[13] Balachandran, R.; ter Haar, E.; Welsh, M. J.; Grant, S. G.; Day, B. W. *Anti-Cancer Drugs* **1998**, *9*, 67.

[14] Martello, L. A.; McDaid, H. M.; Regl, D. L.; Yang, C. H.; Meng, D.; Pettus, T. R. R.;
Kaufman, M. D.; Arimoto, H.; Danishefsky, S. J.; Smith, A. B., III; Horwitz, S. B. *Clin. Cancer Res.* **2000**, *6*, 1978.
[15] ter Haar, E.; Rosenkranz, H. S.; Hamel, H.; Day, B. W. *Bioorganic & Medicinal Chemistry,* **1996**, *4*, 1659.
[16] a) Paterson, I.; Wren, S. P. *J. Chem. Soc., Chem. Commun.* **1993**, 1790. b) Clark, D. L.; Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 5878. c) Golec, J. M. C.; Jones, S. D. *Tetrahedron Lett.* **1993**, *34*, 8159. d) Evans, P. L.; Golec, J. M. C.; Gillespie, R. J. *Tetrahedron Lett.* **1993**, *34*, 8163. e) Golec, J. M. C.; Gillespie, R. J. *Tetrahedron Lett.* **1993**, *34*, 8168. f) Yang, G.; Myles, D. C. *Tetrahedron Lett.* **1994**, *35*, 2503. g) Yang, G.; Myles, D. C. *Tetrahedron Lett.* **1994**, *35*, 1313. h) Paterson, I.; Schlapbach, A. *Synlett* **1995**, 498. i) Miyazawa, M.; Oonuma, S.; Maruyama, K.; Miyashita, M. *Chem. Lett.* **1997**, 1191. j) Miyazawa, M.; Oonuma, S.; Maruyama, K.; Miyashita, M. *Chem. Lett.* **1997**, 1193. k) Marshall, J. A.;Lu, Z. H.; Johns, B. A. *J. Org. Chem.* **1998**, *63*, 817. l) Misske, A. M.;Hoffmann, H. M. R. *Tetrahedron* **1999**, *55*, 4315. m) Evans, D. A.; Halstead, D. P.; Allison, B. D. *Tetrahedron Lett.* **1999**, *40*, 4461. n) Filla,S. A.; Song, J. J.; Chen, L. R.; Masamune, S. *Tetrahedron Lett.* **1999**, *40*,5449.
[17] a) Smith, A. B., III; Qiu, Y. P.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc.* **1995**, *117*, 12011. b) Smith, A. B., III; Kaufman, M. D.; Beauchamp, T. J.; LaMarche, M. J.; Arimoto, H. *Org. Lett.* **1999**, *1*, 1823. Additions and corrections, *Org. Lett.* **2000**, *2*, 1983. c) Smith, A. B., III; Beauchamp, T. J.; LaMarche, M. J.; Kaufman, M. D.; Qiu, Y. P.; Arimoto, H.; Jones, D. R.; Kobayashi, K. *J. Am. Chem. Soc.* **2000**, *122*, 8654.
[18] Harried, S. S.; Yang, G.; Strawn, M. A.; Myles, D. C. *J. Org. Chem.* **1997**, *62*, 6098.
[19] Marshall, J. A.; Johns, B. A. *J. Org. Chem.* **1998**, *63*, 7885.
[20] Paterson, I.; Florence, G. J.; Gerlach, K.; Scott, J. P.; Sereinig, N. *J. Am. Chem. Soc.* 2001, *123,* 9535.
[21] Evans, D. A.; Gauche-Prunet, J. A. *J. Org. Chem.* **1993**, *58*, 2446.
[22] The relative stereochemistry was confirmed by NOE experiments.
[23] Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.
[24] Horita, K.; Yoshioka, T.; Tanaka, T.; Oikawa, Y.; Yonemitsu, O. *Tetrahedron* **1986**, *42*, 3021.
[25] Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 2173.

[26] Gardette, M.; Jabri, N.; Alexakis, A.; Normant, J. F. *Tetrahedron* **1984**, *40*, 2741.

[27] a) Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370. b) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.

[28] a) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem Soc.* **1987**, *109*, 5551. b) Link, J. O. Ph.D. Thesis, Harvard University, 1992.

[29] Yang, G.; Myles, D. C. *Tetrahedron Lett.*, **1994**, *35*, 1313.

[30] Yang, G.; Myles, D. C. *Tetrahedron Lett.*, **1994**, *35*, 2503

[31] Masamune, S.; Ellingboe, J.W.; Choy, W. *J. Am. Chem. Soc.* **1985**, *107*, 1246.

[32] Aldehyde **1'** was prepared from the commercially available (S)-3-hydroxy-2-methylpropionate in 5 steps, see reference 29.

[33] Danishefsky, S. J.; Larson, E.; Askin, D.; Kato, N. *J. Am. Chem. Soc.* **1985**, *107*, 1246.

[34] Jin, H.; Uenishi, J.-I.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*. 5644. (b) Takai, K.; Tagashira, M.; Kuroda, T.; Oshima, K.; Utimoto, Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048.

[35] a) Tsai, D. J.-S.; Matteson, D. S. *Tetrahedron Lett.* **1981**, *22*, 2751. b) Roush, W. R.; Grover, P. T. *Tetrahedron* **1992**, *48*, 1981.

[36] Kocovsky, P. *Tetrahedron Lett.* **1986**, *27*, 5521.

[37] a) Brown, H. C.; Bhat, K. S.; Randad, R. S. *J. Org. Chem.*, **1989**, *54*, 1570 and references therein.

[38] Negishi, E.; Valente, L. F.; Kobayashi, M. *J. Am. Chem. Soc.* **1980**, *102*, 3298.

[39] Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127.

[40] a) Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139. b) Iversen, T.; Bundle, D. R.; *J. Chem. Soc., Chem. Commun.* **1981**, 1240. *23*, 889.

[41] Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.

[42] a) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989. b) Evans, D. A.; Bender, S. L.; Morris, J. *J. Am. Chem. Soc.* **1988**, *110*, 2506.

[43] Parikh, J. R.; Doering, W. von E. *J. Am. Chem. Soc.* **1967**, *89*, 5505.

[44] Penning, T. D.; Djuric, S. W.; Haack, R. A.; Kalish, V. J.; Miyashiro, J. M.; Rowell, B. W.; Yu, S. S. *Synth. Commun.* **1990**, *20*, 307.

[45] Corey, E. J.; Pyne, S. G.; Su, W. -G. *Tetrahedron Lett.* **1983**, *24*, 4883.

[46] Chen, J.; Wang, T.; Zhao, K. *Tetrahedron Lett.* **1994**, *35*, 2827.

[47] a) Smith, A. B., III.; Condon, S. M.; McCauley, J. A. *Acc. Chem. Res.* **1998**, *31*, 35. b) FK506 formal synthesis: Smith, A. B., III; Chen, K.; Robinson, D. J.; Laakso, L. M.; Hale, K. J. *Tetrahedron Lett.* **1994**, *35*, 4271. Also see: Smith, A. B., III; Hale, K. J. *Tetrahedron Lett.* **1989**, *30*, 1037. Smith, A. B., III.; Hale, K. J.; Laakso, L. M.; Chen, K.; Rie´ra, A. *Tetrahedron Lett.* **1989**, *30*, 6963. c) Rapamycin and 27-demethoxyrapamycin total syntheses: Smith, A. B., III; Condon, S. M.; McCauley, J. A.; Leazer, J. L., Jr.; Leahy, J. W.; Maleczka, R. E., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 947. Smith, A. B., III; Condon, S. M.; McCauley, J. A.; Leazer, J. L., Jr.; Leahy, J. W.; Maleczka, R. E., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 962. Smith, A. B., III; Condon, S. M.; McCauley, J. A.; Leazer, J. L., Jr.; Leahy, J. W.; Maleczka, R. E., Jr. *J. Am. Chem. Soc.* **1995**, *117*, 5407. Also see: Smith, A. B., III; Condon, S. M.; McCauley, J. A.; Leahy, J. W.; Leazer, J. L., Jr.; Maleczka, R. E., Jr. *Tetrahedron Lett.* **1994**, *35*, 4907. Smith, A. B., III; Maleczka, R. E., Jr.; Leazer, J. L., Jr.; Leahy, J. W.; McCauley, J. A.; Condon, S. M. *Tetrahedron Lett.* **1994**, *35*, 4911.

[48] Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 889.

[49] Evans, D. A.; Truesdale, L. K.; Grimm, K. G.; Nesbitt, S. L. *J. Am. Chem. Soc.* **1977**, *99*, 5009

[50] Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287.

[51] Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560.

[52] To suppress TBS migration to the primary carbanion, a mixture of **36** and dried $ZnCl_2$, in $Et_2O$, was cooled at -78 °C before treatment with *t* BuLi

[53] Ikeda, Y.; Ukai, J.; Ikeda, N.; Yamamoto, H. *Tetrahedron* **1987**, *43*, 723.

[54] Dauben, W. G.; Gerdes, J. M.; Bunce, R. A. *J. Org. Chem.* **1984**, *49*, 4293.

[55] Takano, S.; Akiyama, M.; Sato, S.; Ogasawara, K. *Chem. Lett.* **1983**, 1593.

[56] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.

[57] Boeckman, R. K, Jr.; Potenza, J. C.; *Tetrahedron Lett.* **1985**, *26*, 1411.

[58] Marshall, J. A.; Adams, N. D. *J. Org. Chem.* **1998**, *63*, 3812.
[59] Marshall, J. A.; Lu, Z.-H.; Johns, B. A. *J. Org. Chem.* **1998**, *63*, 817.
[60] Marshall, J. A.; Xie, S. *J. Org. Chem.* **1995**, *60*, 7230.
[61] Andringa, H.; Keus-Kloos, Y. A.; Brandsma, L. *J. Organomet. Chem.* **1987**, *336*, C41.
[62] Hodgson, D. M.; Wells, C. *Tetrahedron Lett.* **1992**, *33*, 4761.
[63] Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
[64] Bal, B. S.; Childers, W. E.; Pinnick, H. W. *Tetrahedron* **1983**, *37*, 2091.
[65] a) Paterson, I.; Norcross, R. D.; Ward, R. A.; Romea, P.; Lister, M. A. *J. Am. Chem. Soc.* **1994**, *116*, 11287. b) Paterson, I.; Lister, M. A. *Tetrahedron Lett.* **1988**, *29*, 585. c) Paterson, I.; Channon, J. A. *Tetrahedron Lett.* **1992**, *33*, 797. d) Paterson, I.; Tillyer, R. D. *J. Org. Chem.* **1993**, *58*, 4182. e) Paterson, I.; Goodman, J. M.; Isaka, M. *Tetrahedron Lett.* **1989**, *30*, 7121. f) Paterson, I. *Pure Appl. Chem.* **1992**, *64*, 1821.
[66] a) Paterson, I.; Perkins, M. V. *Tetrahedron* **1996**, *52*, 1811. (b) Paterson, I.; Perkins, M. V. *Tetrahedron Lett.* **1992**, *33*, 801.
[67] (a) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945. (b) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511. (c) Rychnovsky, S. D.; Rogers, B.; Richardson, T. I. *Acc. Chem. Res.* **1998**, *31*, 9. (d) Evans, D. A.; Reiger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, *31*, 7099.
[68] a) Carling, R. W.; Holmes, A. B. *J. Chem. Soc., Chem. Commun.* **1986**, 325. b) Curtis, N. R.; Holmes, A. B.; Looney, M. G. *Tetrahedron* **1991**, *47*, 7171. c) Congreve, M. S.; Holmes, A. B.; Looney, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 5815. d) Fuhry, M. A. M.; Holmes, A. B.; Marshall, D. R. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2743. e) Burton, J. W.; Clark, J. S.; Derrer, S.; Stork, T. C.; Bendall, J. G.; Holmes, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 7483. f) Harrison, J.; Holmes, A. B. *Synlett* **1999**, 972.
[69] Paterson, I.; Wallace, D. J.; Cowden, C. J. *Synthesis* **1998**, 639.
[70] a) Cintas, P. *Synthesis* **1992**, 248. b) Hodgson, D. M.; Wells, C. *Tetrahedron Lett.* **1992**, *33*, 4761. c) Andringa, H.; Heus Kloos, Y. A.; Brandsma, L. *J. Organomet. Chem* **1987**, *336*, C41.
[71] For a review of Peterson-type eliminations, see: Ager, D. *Org. React.* **1990**, *38*, 1.
[72] Hall, P. L.; Gilchrist, J. H.; Collum, D. B. *J. Am. Chem. Soc.* **1991**, *113*, 9571.

[73] Kobayashi, S.; Tsuchiya, K.; Harada, T., Nishide, M.; Kurokawa, T.; Nakagawa, T.; Shimada, N.; Kobayashi, K. *J. Antibiot*, **1994**, *47*, 697.
[74] Kobayashi, S.; Tsuchiya, K.; Kurokawa, T.; Nakagawa, T.; Shimada, N. *J. Antibiot*, **1994**, *47*, 703.
[75] Yasui, K.; Tamura, Y.; Nakatani, T.; Horibe, I.; Kawada, K.; Koizumi, K.; Suzuki, R.; Ohtani, M. *J. Antibiot*, **1996**, *49*, 173-180.
[76] Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikawa, K.; Nishikiori, T.; Mayumi, T.; Osada, H. *Cancer Lett*, **1998**, *126*, 29-32
[77] Yasui, K.; Tamura, Y.; Nakatani, T.; Kawada, K.; Ohtani, M. *J. Org. Chem.* **1995**, *60*, 7567-7574.
[78] Kobayashi, S.; Tsuchiya, K.; Nishide, M.; Nishikiori, T.; Nakagawa, T.; Shimada, N. *J. Antibiot*, **1995**, *48*, 893-895.
[79] a) Fraser-Reid, B.; Boctor, B. *Can. J. Chem.* **1969**,*47*, 393. b) Morimoto, Y.; Mikami, A.; Shirahama, H. *J. Chem. SOC., Chem. Commu.* **1991,** 1376.
[80] a) Corey, E. J.; Weigel, L. *0.;* Chamberlin, **A.** R.; Lipshutz, B, *J . Am. Chem. Soc.* **1980**,*102*, 1439. b) Tatsuta, K.: Kowchi, Y.; Kase, M. *Bull. Chem. Soc. Jpn.* **1988,** *61*, 2525.
[81] a) Hutchins, R. O.; Kacher, M.; Rua, L. *J. Org. Chem.* **1975**, *40*, 923 - 926. b) Hutchins, R. O.; Natale, N.R. *J. Org. Chem.* **1978**, *43*, 2299-2301.
[82] a) Tipson, R. S.; Cohen, **A.** *Carbohydr. Res.* **1965**, *1*, 338. b) Fraser-Reid, B.; Boctor, B. *Can. J. Chem.* **1969**, *47*, 393. c) Holder, N. L.; Fraser-Reid, B. *Can. J. Chem.* **1973**, *51*, 3357. For a general review, see: d) Block, E. *Org. React.* **1984**, ***30***, 457.
[83] Gurjar, M, K.; Henri Jr, J. T.; Bose, D. S.; Rama Rao, A. V. *Tetrahedron Letters* **1996**, *37*,
6615-6618
[84] Gurjar, M, K.; Chakrabarti, A.; Rama Rao, A. V. *Heterocycles*, **1997**, *45*, 7-10.
[85] Rama Rao, A. V.; Murali Dhar, T. J.; Bose, D. S.; Chakrabarti, A.; Gurjar, M, K. Tetrahedron **1989**, *45*, 7361-7370.
[86] Katsuki, T.; Martin, V. S. *Organic reactions*
[87] a) Evans, D. A.; Miller, S. J.; Ennis, M. D.; Ornstein. P. L. *J. Org. Chem.* **1992**, *57*, 1067-1069. b) Evans, D. A.; Rieger, D. L.; Bilodeau, M. T.; Urpipp, F. *J. Am. Chem. Soc.* **1991**, *113*,1047-1049.
[88] Reddy, M. A.; Gopal, R. J.; Rao, V. J. Indian J. Chem, **1996**, *35B*, 312.

[89] Hirai, Y.; Chintani, M.; Yamazaki, T.; Momose, T. *Chem. Lett,* **1989**, 1149.
[90] Brown, H. C.; Yoon, N. M. J.Am. Chem. Soc, **1968**, *90*, 2686-2688.
[91] a) Schlosser, M.; Christmann, K.F. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 689. b) Schlosser, M.; Christmann, K.F. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 126. c) Rama Rao, A. V. Gurjar, M. K.; Bose, D. S.; Revathi Devi, R. *J. Org. Chem.* **1991**, *56*, 1320-1321.
[92] Chida, N.; Yoshinaga, M.; Tobe, T.; Ogawa, S. *Chem. Commun.*, **1997**, 1043-1044.
[93] Utilisation of l-quebrachitol in organic synthesis, see; a) Chida, N.; Yamada, K.; Ogawa, S. *J. Chem. Soc., Perkin Trans. 1*, **1993**, 1957; b) Chida, N.; Ogawa, S. *Chem. Commun,* **1997**, 807; c) Kiddle, J. J. *Chem. Rev*, **1995**, *95*, 2189.
[94] a) Tsuda, Y.; Nishimura, M.; Kobayashi, T.; Sato Y.; Kanemitsu, K. Chem. Pharm. Bull, **1991**, *39*, 2883; b) David, S.; Hanessian, S.; *Tetrahedron,* **1985**, *41*, 643-663.
[95] Takai, K.; Nitta, K.; Utimoto K. *J. Am. Chem. Soc* **1986**, *108*, 7408; b) Okazoe, T.; Takai, K.; Utimoto, K. *J. Am. Chem. Soc.*, **1987**, *109*, 951.
[96] Hughes, L. *Org. React,* **1993**, *42*, 656.
[97] a) Nakata, M.; Ishiyama, T.; Akamatsu, S.; Hirose, Y.; Maruoka, H.; Suzuki, R.; Tatsuta, K. *Bull. Chem. Soc. Jpn.*, 1995, **68**, 967; b) Wasmuth, D.; Arigoni, D.; Seebach, D. *Helv. Chim. Acta*, **1982**, *65*, 344.
[98] Cheng, Y.-S.; Liu, W.-L.; Chen, S. *Synthesis*, **1980**, 223.
[99] Nicolaou, K. C.; Ajito, K.; Patron, A. P.; Khatuya, H.; Richter, P. K.; Bertinato, P. *J. Am. Chem. Soc*, **1996**, *118*, 3059-3060.
[100] a) Corey, E. J.; Erickson, B. W. *J. Org. Chem,* **1971**, *36*, 3553; (*b*) Shimizu, S.; Nakamura, S.; Nakada, M.; Shibasaki, M. *Tetrahedron*, **1996**, *52*, 13363-13408.
[101] Saksena, A. K.; Magiaracina, P. *Tetrahedron Lett.*, **1983**, *24*, 273.
[102] Watanabe, H.; Watanabe, H.; Kitahara, T. *Tetrahedron Lett*, **1998**, *39*, 8313-8316.
[103] Watanabe, H.; Watanabe, H.; Bando, M.; Kido, M.; Kitahara, T. *Tetrahedron.*, **1999**, *55*, 9755-9776
[104] Honda, M.; Katsuki, T.; Yamaguchi, M. *Tetrahedron Lett*, **1984**, *25*, 3857.
[105] a) Kitahara, T.; Mori, K. *Tetrahedron Lett*, **1985**, *26*, 451; b) Kitahara, T. kurata, H.; b) Mori,K. *Tetrahedron*, **1988**, *44*, 4339.

[106] Mori, Y.; Suzuki, K. *Tetrahedron Lett*, **1989**, *30*, 4383.
[107] Keck, G. E.; Knutson, C. E.; Wiles, S. A. *Org. Lett*, **2001**, *3*, 707 – 710.
[108] Keck, G. E.; Li, X. -Y.; Knutson, C. E. *Org. Lett*, **1999**, *1*, 411-414.
[109] a) Keck, G. E.; *Tetrahedron Lett*, **1984**, *25*, 1883, b) Keck, G. E.; Savin, K. A.; Cressman, E. N. K.; Abbott, D. E. *J. Org. Chem*, **1994**, *59*, 7889.
[110] Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett*, **1972**,*13*, 3769.
[111] Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis*, **1994**, 639.
[112] Myers, A. G.; Yang, B. H.; Chen, H.; McKinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc*, **1997**, *119*, 6496 - 6511.
[113] Keck, G. E.; Wager, C. A.; Sell, T.; Wager, T. T. *J. Org. Chem*, **1999**, *64*, 2172.
[114] Dias, L. C.; de Oliveira, L. G.; de Sousa, M. A. *Org. Lett.* **2003**, *5*, 265 - 268
[115] a) Ando, K. *J. Org. Chem.* **1997**, *62*, 1934; b) Ando, K. *J. Chem. Soc.* **1998**, *63*, 8411; c) Ando, K. *J. Org. Chem.* **1999**, 64, 8406; d) Ando, K.; Oishi, T.; Hirama, M.; Ohno, H.; Ibuka, T. *J. Org. Chem.* **2000**, *65*, 4745.

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# Antitumor and Vascular Physiological Effects of Natural Products

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**ABSTRACT:** It has recently been reported that cancer, arteriosclerosis and coronary heart diseases have long been considered major contributors to morbidity and mortality in developed countries, such as Western Europe, United States of America and Japan. Although a number of medicinal plants and foodstuffs are used for their preventive effects against life-style-related diseases, such as coronary heart diseases, hypertension, thrombosis, allergic inflammation, arteriosclerosis, diabetes and cancer, the basis for clinical studies and experimental evidence are insufficient and unclear. Therefore, to clarify whether natural products have preventive effects on tumors, tumor metastasis, allergic inflammation and coronary artery constriction, I used biochemical and pharmacological approaches to study the natural products isolated from various medicinal plants and foodstuffs. In the present review, I have introduced the biological and pharmacological actions of various components, isolated from some medicinal plants and foodstuffs, on tumors and tumor metastasis in tumor-bearing mice, and on adhesion molecule expression and artery constriction in vascular endothelium. Fucoidan (polysaccharides), carp oil (fatty acids) and triterpenoids inhibited the tumor growth and/or metastasis in the liver, through the inhibition of tumor-induced neovascularization, in tumor-bearing mice. Baicalein (flavones) isolated from *Scutellaria baicalensis* roots inhibited the thrombin-induced adhesion molecule through the inhibition of protein C kinase activation. Furthermore, xanthoangelol (chalcones) isolated from *Angelica keiskei* roots inhibited catecholamine-induced vasoconstriction through endothelium-dependent nitric oxide production.

## INTORODUCTION

Recently, it has been reported that cancer, arteriosclerosis and coronary heart diseases have long been considered major contributors to morbidity and mortality in developed countries. Many medicinal plants and foodstuffs are used for their preventive effects against life-style-related diseases, such as coronary heart diseases, hypertension, thrombosis, allergic inflammation, arteriosclerosis, hyperlipidemia, and cancer. Although based on the classic concept of "Diet and Medicine from the Same Source", as described in old Chinese and Japanese medical books, evidence based on clinical and experimental results were not yet clear. I have been using biochemical and pharmacological approaches to study

many natural products isolated from various medicinal plants and foodstuffs for about 25 years. In a previous review [1], I described the combined effects of natural products, such as chitosan and fish oils (tuna and carp oil), and cancer chemotherapy drugs, such as cisplatin and doxorubicin, on tumor growth and adverse reactions in tumor-bearing mice. Recently, I found that fucoidan isolated from *Laminaria japonica*, triterpenoid fractions isolated from *Ganoderma lucidum*, carp oil, and oleic acid, had antitumor and/or antimetastatic activities in tumor-bearing mice. Furthermore, I found that flavonoids, isolated from the roots of *Scutellaria baicalensis,* inhibited thrombin- or thrombin receptor agonist peptide-induced adhesion molecule expression in human umbilical vein endothelial cells (HUVECs), and that various chalcones isolated from the roots of *Angelica keiskei,* inhibited catecholamine-induced vasoconstriction in rat aortic rings. In the present review, I have introduced the antitumor and vascular physiological effects of these natural products.

## 1. Antitumor Effects of Natural Products

### a) *Antitumor Activity of Fucoidan Isolated from Laminaria japonica and Its Mechanism* [2]

Brown algae of the *Laminaria* species (Phaeophyceae) are widely used in Japan for food and relishes, while *Laminaria japonica* Aresch (Japanese name: Makonbu) is employed as a Chinese and Japanese traditional medicine for the treatment and prevention of hyperlipidemia, arteriosclerosis, and cancer. Brown algae contain large quantities of alginic acid and fucoidan. I studied the effects of fucoidan isolated from *L. japonica* on tumor growth in sarcoma 180-bearing mice. As shown in "Fig. (**1**)", tumor growth and final tumor weight in sarcoma 180-bearing mice were inhibited by the oral administration of fucoidan at a dose of 300 mg/kg for 14 consecutive days. Fucoidan had no effect on body, thymus, spleen or adipose tissue weight "Table (**1**)".

These findings indicated that fucoidan did not cause adverse reactions such as body weight loss, gastrointestinal toxicity or the immunotoxicity induced by cancer chemotherapy drugs, such as 5-fluorouracil, cisplatin and doxorubicin. Angiogenesis is the growth of new capillary blood vessels from preexisting capillaries and post capillary venules. Solid tumors cause neovascularization, and the resultant angiogenesis from the solid tumors stimulates growth. To clarify the antitumor effects of fucoidan, I examined the effects of fucoidan on angiogenesis induced by Matrigel and acidic fibroblast growth factor (a FGF). Fucoidan (150 and 300 mg/kg x 2 /day) inhibited the angiogenesis induced Matrigel and aFGF as shown in "Table (**2**)" and Fig, (**2**)". These results suggest that the antitumor activity of fucoidan, isolated from *L. japonica,* may be due to the inhibition of the angiogenesis induced by tumors.

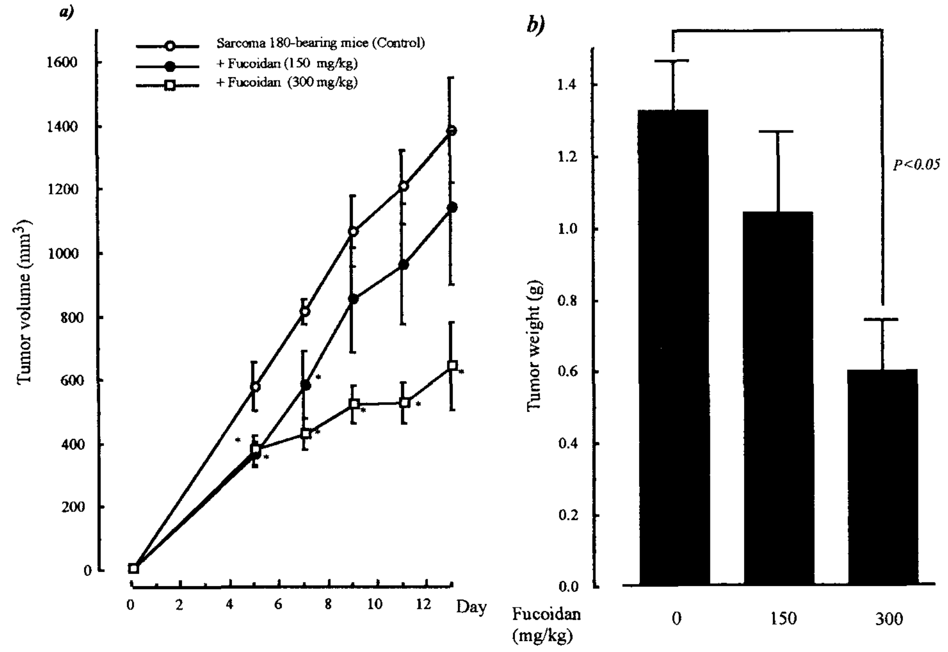



Fig. (1). Effects of fucodian on tumor growth (a), and final tumor weight (b), in sarcoma 180-bearing mice. Fucoidan (150 or 300 mg/kg) was administered orally for 14 days. On day 15, blood and various tissues were obtained. Results are expressed as means ± S.E. of 10 mice.

**Table 1. Effects of fucoidan on body, thymus, spleen and adipose tissue weight in sarcoma 180-bearing mice[1]**

| | No of animals | Final body weight (g) | Thymus (mg) | Spleen (mg) | Adipose tissue (mg) |
|---|---|---|---|---|---|
| Sarcoma-180-bearing mice (Control) | 10 | 37.5 ± 0.48 | 71.0 ± 5.00 | 178.1 ± 9.03 | 538.2 ± 36.2 |
| + Fucoidan (150 mg/kg) | 10 | 36.6 ± 0.81 | 75.2 ± 5.12 | 150.0 ± 12.2 | 621.4 ± 66.8 |
| (300 mg/kg) | 10 | 37.1 ± 0.63 | 63.8 ± 4.97 | 149.3 ± 11.3 | 520.1 ± 39.7 |

[1]Values are expressed as means ± S.E. of 10 mice in each group.

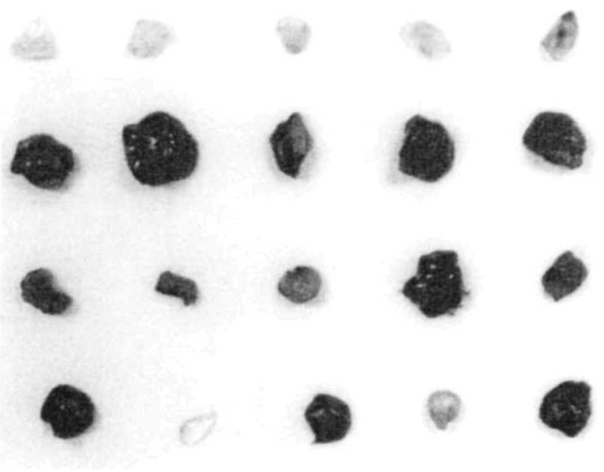



**Fig. (2).** Photograph of Matrigel, 5 days after a subcutaneous injection with 0.5 mL Matrigel alone (a), Matrigel supplemented with aFGF and heparin without (b), or with 150 mg/kg (c), or 300 mg/kg (d) of fucoidan administered orally twice from day 0 to 4.

**Table 2. Effects of fucoidan on angiogenesis induced by Matrigel supplemented with aFGF and heparin[1]**

| Treatment | Matrigel weight (mg) | Hemoglobin (mg/Matrigel) |
|---|---|---|
| Matrigel alone | 83.3 ± 8.71* | 8.2 ± 0.58* |
| Matrigel + aFGF (1 ng/mL) + heparin (64 U/mL) | 400.8 ± 72.3 | 21.8 ± 3.93 |
| Matrigel/aFGF/heparin | | |
| + Fucoidan (150 mg/kg x 2/day) | 191.9 ± 53.7* | 12.5 ± 1.41* |
| (300 mg/kg x 2/day) | 145.0 ± 45.4* | 13.6 ± 1.36* |

[1]C57BL/6 mice were injected subcutaneously with 0.5 mL of Matrigel supplemented with aFGF and heparin. The mice were sacrificed on day 5 with an overdose of pentobarbital, and the gels were removed and weighed. Values are expressed as means ± S.E. of 5 mice. * $P<0.05$, Significantly different from Matrigel/aFGF/heparin mixture-treated mice.

### *b) Antitumor and antimetastatic effects of the triterpenoids of Ganoderma lucidum; Its mechanism of Action and Isolation of An Active Substance [3]*

The fruit body of *Ganoderman lucidum* (Fr.) Karst has been used for the treatment of hypertension, hyperlipidemia, arthritis, bronchitis, arteriosclerosis, diabetes and cancer. I previously reported that an aqueous extract of *G. lucidum* reduced the elevation of blood glucose without elevating blood insulin in a test utilizing an intravenous infusion of adrenaline and an oral infusion of glucose [4]. The polysaccharide

fractions of *G. lucidum* have potent antitumor activities in tumor-bearing animals [5-9]. Recently, Min et al. [10] reported that triterpenoids isolated from the spores of *G. lucidum* have cytotoxicity against Meth-A and Lewis lung carcinoma (LLC) *in vitro*. However, the antitumor and antimetastatic activities of the triterpenoid fraction of *G. lucidum* had not yet been studied in *in vivo* animal models. In the present review, I have introduced the antitumor and antimetastatic effects of triterpenoids in mice with intrasplenically implanted LLC. As shown in "Fig. (**3**)", triterpenoid fractions (100 or 200 mg/kg) inhibited the primary tumor weight in spleens of mice with intrasplenically implanted LLC. Moreover, the triterpenoid fractions, at a dose of 100 or 200 mg/kg, significantly reduced the number of tumor cell colonies that metastasized in the liver, compared with the number intrasplenically implanted mice "Fig. (**4**)".

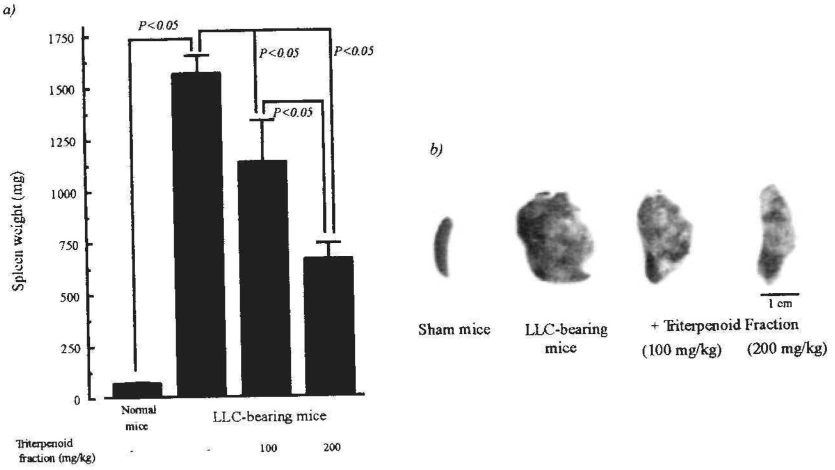
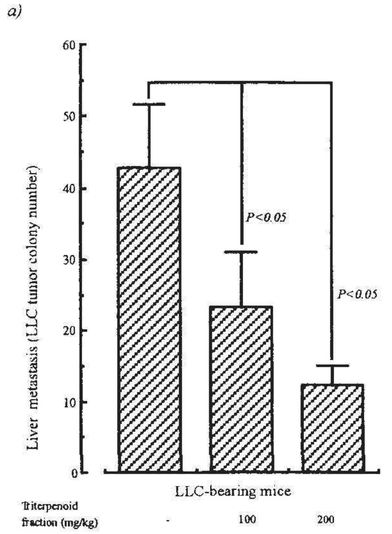



**Fig. (3).** Antitumor effects of triterpenoid fractions of *G. lucidum* in mice with intrasplenically implanted LLC.

a) Effects of triterpenoid fractions on primary tumors in the spleen. Results are expressed as means ± S.E. of 5-8 mice in each group. The sham-operated group consisted of five mice; the LLC-bearing group (control) and triterpenoid fraction-treated group both consisted of eight mice.

b) Photographs of the inhibition of intrasplenic tumors by triterpenoid fractions.

The number of leukocytes in LLC-bearing mice was significantly greater than that in normal mice. In contrast, the red cell number and hemoglobin content were significantly lower than those of normal mice "Table (**3**)". The reduction in red cell number and hemoglobin content was prevented by orally administered triterpenoid fractions (100 or 200 mg/kg) "Table (**3**)".



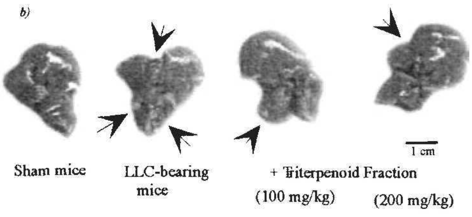




**Fig. (4).** Antimetastatic effects of triterpenoid fractions of *G. lucidum* in mice with intrasplenically implanted LLC.

a) Effects of triterpenoid fraction on the number of colonies of LLC cells metastasizing in the liver. Results are expressed as means ± S.E. of 5-8 mice in each group.

b) Photographs of the inhibition of LLC tumor metastasis to the liver by triterpenoid fraction. Tumors are indicated by arrows.

**Table 3. Effects of triterpenoid fraction on the number of leukocytes and red cells and the hemoglobin content in mice with intrasplenically implanted Lewis lung carcinoma (LLC)[1]**

| | No of animals | Leukocytes ($\times 10^3/\mu L$) | Red cells ($\times 10^4/\mu L$) | Hemoglobin (g/100 mL) |
|---|---|---|---|---|
| Sham-operated mice | 5 | 2.18± 0.31* | 755.4± 9.99* | 11.74± 0.16* |
| LLC-bearing mice | 8 | 5.63± 0.52 | 430.6± 55.24 | 6.56± 0.87 |
| + Triterpenoid | | | | |
| fraction (100 mg/kg) | 8 | 6.03± 0.76 | 579.4± 28.78* | 8.83± 0.44* |
| (200 mg/kg) | 8 | 4.48± 0.49 | 620.1± 37.56* | 9.46± 0.59* |

[1]Values are expressed as means ± S.E. of 5-8 mice. *P<0.05, Significantly different from LLC-bearing mice.

As shown in "Fig. (5)", primary tumor cells in the spleen grew and invaded the white and red pulp of the spleen in mice with intrasplenically implanted LLC. The orally administered triterpenoid fractions (100 or 200 mg/kg) prevented the tumor growth and the invasion of the white pulp of the spleen. Furthermore, the orally administered triterpenoid fractions induced an accumulation of lymphocytes around tumor cells in the white pulp of the spleen "Fig. (5)". The metastatic tumor cells in the liver also grew and invaded the hepatocytes in mice with intrasplenically implanted

LLC. The growth of the metastatic tumor cells was prevented by the orally administered triterpenoid fractions "Fig. (**6a**)". Lymophocytes in the liver also accumulated around the metastatic tumor cells "Fig. (**6b**)".

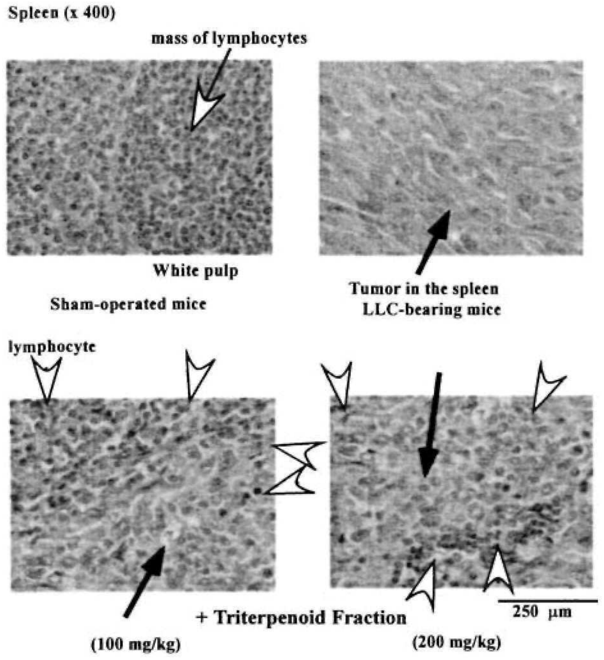



**Fig. (5).** Histology of the inhibition of intrasplenic tumors (x 400) by triterpenoid fractions in mice with intrasplenically implanted LLC.
Tumors, closed arrows; lymphocytes, open arrows.

To clarify the antitumor effects of triterpenoids, I examined the effects of triterpenoids on DNA synthesis in LLC cells (*in vitro*), and on angiogenesis induced by Matrigel in the presence of vascular endothelial cell growth factor (VEGF) (*in vivo*). Triterpenoid fractions had no effect on DNA synthesis in LLC cells (data not shown). As shown in "Fig. (**7**)", the gels that formed after the subcutaneous implantation of Matrigel alone, were readily distinguished from the surrounding tissue and produced little or no local reaction or angiogenic response "Fig. (**7b**)". However, gels produced from Matrigel supplemented with 20 ng/mL VEGF and 32 U/mL heparin showed an angiogenic reaction "Fig. (**7b**)". Use of the Matrigel/VEGF/heparin mixture significantly increased the weight of the gels and the hemoglobin content in the gels at 6 days after implantation, compared with those in mice injected with Matrigel alone. The triterpenoid fraction (800 µg/mL), and the acidic fraction (800 µg/mL) purified from the triterpenoid fractions, significantly inhibited the angiogenesis induced by the Matrigel/VEGF/heparin mixture "Table (**4**)".

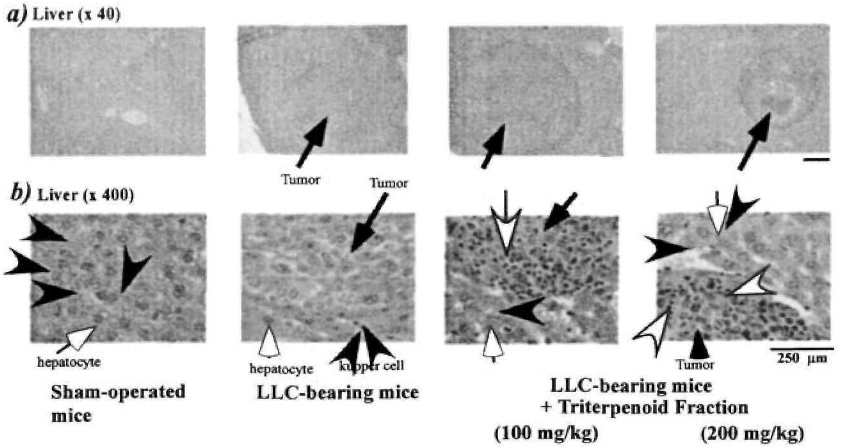



**Fig. (6).** Histology of the inhibition of the metastasis of tumor cells in the liver (x 40) (a), and (x 400) (b), by triterpenoid fractions in mice with intrasplenically implanted LLC. Tumors, closed arrows; lymphocytes, open arrows; kupper cells, closed rhombi; hepatocytes, open circles.

**Table 4. Effects of triterpenoid fractions, and acidic and neutral fractions, isolated from triterpenoid fractions of *Ganoderma lucidum*, on angiogenesis induced by Matrigel supplemented with VEGF and heaprin[1]**

| Treatment | No of animals | Matigel weight (mg) | Hemoglobin (mg/Matrigel) |
|---|---|---|---|
| Matrigel alone | 4 | 116.0 ± 9.79* | 5.8± 1.93* |
| Matrigel + VEGF (20 ng/mL) + heparin (32 U/mL) | 4 | 373.3 ± 9.21 | 23.8 ± 2.25 |
| Matrigel/VEGF/heparin + Triterpenoid fraction (800 μg/mL) | 4 | 252.6 ± 19.55* | 13.3 ± 3.18* |
| Matrigel/VEGF/heparin + Acidic fraction (800 μg/mL) | 4 | 178.8 ± 25.44* | 10.3 ± 1.20* |
| Matrigel/VEGF/heparin + Neutral fraction (800 μg/mL) | 4 | 427.1 ± 99.08 | 28.3 ± 7.97 |

[1]C57BL/6 mice were injected subcutaneously with 0.5 mL of Matrigel, supplemented with VEGF and heparin, in the presence or absence of various fractions. The mice were sacrificed on day 5 with an overdose of pentobarbital, and the gels were removed and weighed. Values are expressed as means ± S.E. of 4 mice.

* $P<0.05$, Significantly different from Matrigel/VEGF/heparin mixture-treated mice.

Furthermore, the isolation of the active substance(s) from the acidic fraction of the triterpenoid fractions, was performed using the Matrigel-implanted angiogenesis assay, and seven fractions were obtained from the acidic fraction by silica gel column chromatography.

Among these seven fractions, fractions 2 and 3 inhibited the angiogenesis

"Table (5)". Compound 1 was isolated from fractions 2 and 3 as an angiogenic substance by repeated silica gel column chromatography. Compound 1, at a dose of 800 μg/mL, inhibited the angiogenesis "Fig. (7a)".

**Table 5. Effects of various fractions isolated from acidic fraction of *G. lucidum* on angiogenesis induced by Matrigel supplemented with VEGF and heparin[1]**

| Treatment | No of animals | Matigel weight (mg) | Hemoglobin (mg/Matrigel) |
|---|---|---|---|
| Matrigel alone | 3 | 100.0 ± 28.9* | 11.7 ± 0.3* |
| Matrigel + VEGF (20 ng/mL) + heparin (32 U/mL) | 3 | 295.0 ± 16.5 | 25.0 ± 7.0 |
| Matrigel/VEGF/heparin | | | |
| + Fraction 1 (800 μg/mL) | 3 | 250.0 ± 69.3 | 24.3 ± 7.8 |
| + Fraction 2 (800 μg/mL) | 3 | 140.7 ± 74.5* | 21.3 ± 0.9 |
| + Fraction 3 (800 μg/mL) | 3 | 176.7 ± 27.8* | 12.7 ± 0.3* |
| + Fraction 4 (800 μg/mL) | 3 | 210.0 ± 31.1 | 21.0 ± 0.3 |
| + Fraction 5 (800 μg/mL) | 3 | 270.0 ± 41.6 | 28.7 ± 4.3 |
| + Fraction 6 (800 μg/mL) | 3 | 243.3 ± 60.6 | 22.0 ± 4.5 |
| + Fraction 7 (800 μg/mL) | 3 | 280.0 ± 30.0 | 27.5 ± 2.5 |

[1]Values are expressed as means ± S.E. of 3 mice in each group. * P<0.05, Significantly different from Matrigel/VEGF/heparin mixture-treated mice

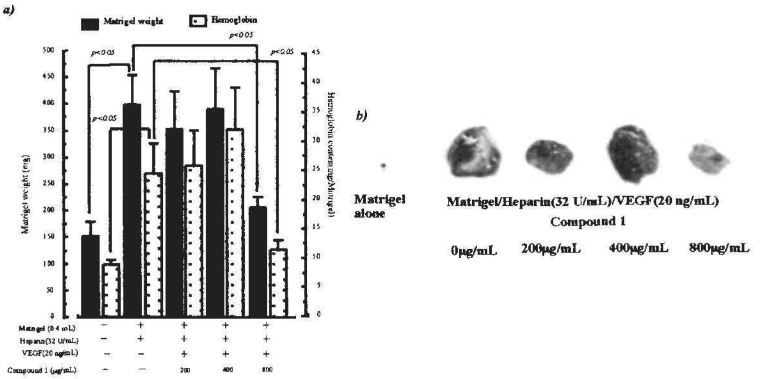



**Fig. (7).** Antiangiogenic effects of compound 1 (ganoderic acid F) isolated from triterpenpoid farctions of *G. lucidum*.

a) Effects of compound 1 (ganoderic acid F) on Matrigel weight and hemoglobin concentgration of gels, induced by Matrigel supplemented with heparin and VEGF. Results are expressed as means ± S.E< of 5 mice in each group.

b) Photographs of Matrigel, 6 days after subcutaneously injection of Matrigel alone, or Matrigel supplemented with heparin and VEGF, in the absence or presence of the indicated amounts of compound 1 (ganoderic acid F).

Compound 1 was identified as ganoderic acid F based on the data of IR, $^1H$- and $^{13}C$-NMR, and MS analyses "Fig. (**8**)". Therefore, these findings suggest that the mechanism of inhibition of primary solid-tumor growth, metastasis in the liver, and secondary metastatic tumor growth, by the triterpenoid fractions, might partly be due to the inhibition of angiogenesis and the activation of immunofunction.

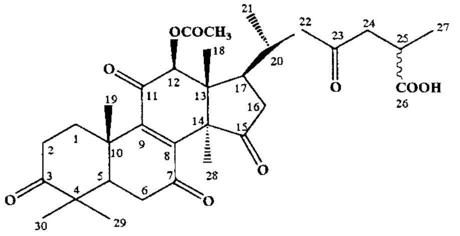



**Fig. (8).** Ganoderic acid F (12-acetoxy-3,7,11,15,23-pentaoxolanost- 8en-oic acid).

*c) Carp Oil or Oleic Acid Inhibits Tumor Growth and Metastasis in Mice with Intrasplenically Implanted LLC* [11]

The fresh water carp (*Cyprinus carpio* L.) has long been used in Korea, China and Japan as a health food. In ancient Chinese medicine, carp was eaten as a diuretic and used as a remedy for eye fatigue. In Japan, carp meat and blood have traditionally been eaten as a tonic. Although it has recently been thought that carp oil prevents the recurrence of tumors and metastasis after stopping radiation and/or cancer chemotherapy, the basis for this is unclear. In this review, I have introduced the antitumor and antimetastatic activities of carp oil, and the oleic acid in carp oil, in mice with intrasplenically implanted LLC. As shown in "Fig. (**9**)", the spleen weights of mice with intrasplenically implanted LLC were significantly greater than those of sham-operated mice. In LLC-bearing mice, the increase in spleen weight was significantly inhibited by the oral administration of carp oil at a dose of 0.1 or 0.2 mL per mouse "Fig. (**9**)". The composition of the fatty acids in carp oil is shown in "Table (**6**)", and oleic acid makes up about 37% of the oil.

On the other hand, oleic acid (0.1 or 0.2 mL per mouse) did not inhibit tumor growth in the spleen "Table (**7**)". Carp oil (0.2 mL per mouse), and oleic acid (0.1 or 0.2 mL per mouse), significantly reduced the number of tumor cell colonies that metastasized in the liver compared with the number in intrasplenic LLC-bearing mice, as shown in "Fig. (**10**) and Table (**7**)". The growth of the metastatic tumors in the livers of LLC-bearing mice was also inhibited by orally administered carp oil or oleic acid at 0.1 or 0.2 mL per mouse "Fig. (**11**)".

The number of leukocytes in the LLC-bearing mice was significantly greater than in the sham-operated mice.

**Table 6. The fatty acid components of carp oil**

| Fatty acid component | g/kg carp oil |
|---|---|
| Myristic acid, 14:0 | 20 |
| Palmitic acid, 16:0 | 238 |
| Plamitoleic acid, 16:1 (n-7) | 78 |
| Srearic acid, 18:0 | 33 |
| Oleic acid, 18:1 (n-9) | 366 |
| Linoleic acid, 18:2 (n-6) | 182 |
| EPA, 20:5 (n-3) | 9 |
| DHA, 22:6 (n-3) | 23 |
| Others | 51 |

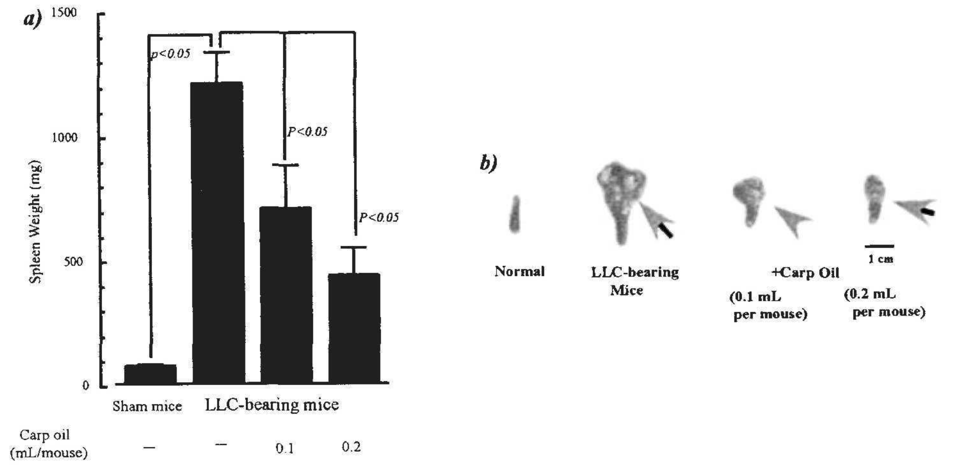



**Fig. (9).** Antitumor effects of carp oil in mice with intrasplenically implanted LLC.

a) Effects of carp oil on final primary solid tumor growth in the spleen in mice with intrasplenically implanted LLC. Results are expressed as means ± S.E. of 5-8 mice. The sham-operated group consisted of five mice; the LLC-bearing group (control) and carp oil-treated group both consisted of eight mice.

b) Photographs of the inhibition of intrasplenic tumor growth on day 21 by carp oil in mice with intrasplenically implanted LLC. Tumors are indicated with arrows.

In contrast, the number of red cells and the hemoglobin concentration in LLC-bearing mice were significantly lower than in the normal mice "Table (**8**) and Table (**9**)", that is, the intrasplenic implantation of LLC cells caused anemia. The increase in leukocyte number in the LLC-bearing mice was inhibited by the orally administered carp oil (0.1 or 0.2 mL per mouse) or oleic acid (0.1 or 0.2 mL per mouse) "Table (**8**) and Table (**9**)". Moreover, oleic acid at 0.1 or 0.2 mL per mouse inhibited the reduction of red cell numbers and hemoglobin concentration in the LLC-bearing mice "Table (**9**)".

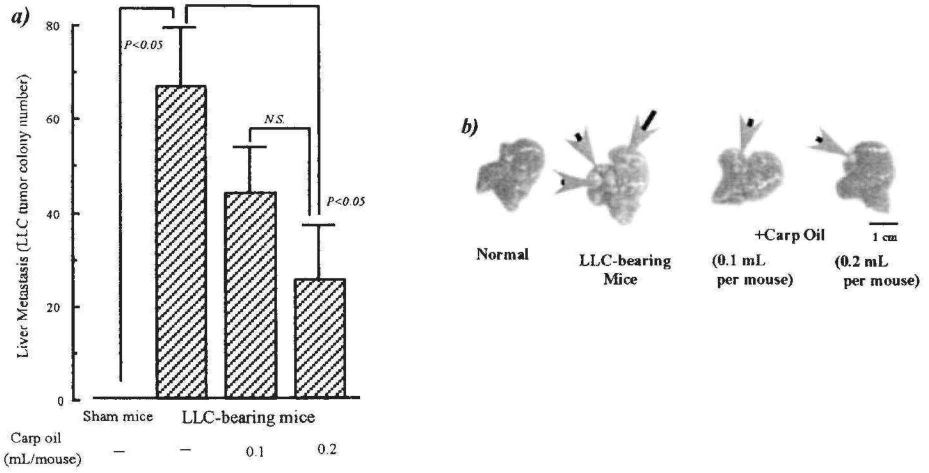



**Fig. (10).** Antimetastatic effects of carp oil in mice with intrasplenically implanted LLC.

a) Effects of carp oil on the number of colonies of LLC cells metastasizing to the liver in mice with intrasplenically implanted LLC. Results are expressed as means ± S.E. of 5-8 mice.

b) Photographs of the inhibition of LLC tumor metastasis in the liver on day 21 by carp oil in LLC-bearing mice. Tumors are indicated with arrows.

**Table 7. Effects of oleic acid on the spleen weight and number of metastatic tumor colonies in the livers of mice with intrasplenically implanted LLC[1]**

| Treatment | No of animals | Spleen weight (mg) | Metastatic tumor colonies in the liver |
|---|---|---|---|
| Sham-operated mice | 5 | 79.1 ± 7.08* | 0 ± 0* |
| LLC-bearing mice | 6 | 504.9 ± 97.6 | 47 ± 12 |
| + Oleic acid (0.1 mL/mouse) | 6 | 334.4 ± 100.4 | 15 ± 6* |
| (0.2 mL/mouse) | 6 | 541.3 ± 172.1 | 24 ± 4* |

[1]Values are expressed as means ± S.E. of 5-6 mice in each group. *P<0.05, Significantly different from LLC-bearing mice.

**Table 8. Effects of carp oil on the numbers of leukocytes, red cells, and hemoglobin concentration in LLC-bearing mice[1]**

| | No of animals | Leukocytes (x $10^3/\mu L$) | Red cells (x $10^4/\mu L$) | Hemoglobin (g/100 mL) |
|---|---|---|---|---|
| Sham-operated mice | 5 | 2.48 ± 0.23* | 786.6 ± 8.23* | 12.1 ± 0.12* |
| LLC-bearing mice | 8 | 7.08 ± 1.83 | 579.1 ± 52.9 | 9.10 ± 0.79 |
| + Carp oil | | | | |
| (0.1 mL/mouse) | 8 | 3.80 ± 0.22* | 572.6 ± 61.7 | 8.90 ± 0.96 |
| (0.2 mL/mouse) | 8 | 3.40 ± 0.34 | 653.6 ± 45.0 | 10.3 ± 0.73 |

[1]Values are expressed as means ± S.E. of 5-8 mice. *P<0.05, Significantly different from LLC-bearing mice.

**Table 9. Effects of oleic acid on the numbers of leukocytes, red cells, and the hemoglobin concentration in LLC-bearing mice[1]**

| | No of animals | Leukocytes ($x 10^3/\mu L$) | Red cells ($x 10^4/\mu L$) | Hemoglobin (g/100 mL) |
|---|---|---|---|---|
| Sham-operated mice | 5 | 3.44 ± 0.89* | 770.6 ± 17.6* | 11.8 ± 0.19* |
| LLC-bearing mice | 6 | 6.22 ± 1.25 | 303.2 ± 64.4 | 4.60 ± 1.00 |
| + Oleic acid | | | | |
| (0.1 mL/mouse) | 6 | 3.26 ± 0.14* | 487.4 ± 45.9* | 7.40 ± 0.72* |
| (0.2 mL/mouse) | 6 | 3.78 ± 0.49 | 567.3 ± 59.4* | 8.90 ± 0.96* |

[1]Values are expressed as means ± S.E. of 5-6 mice. *P<0.05, Significantly different from LLC-bearing mice.

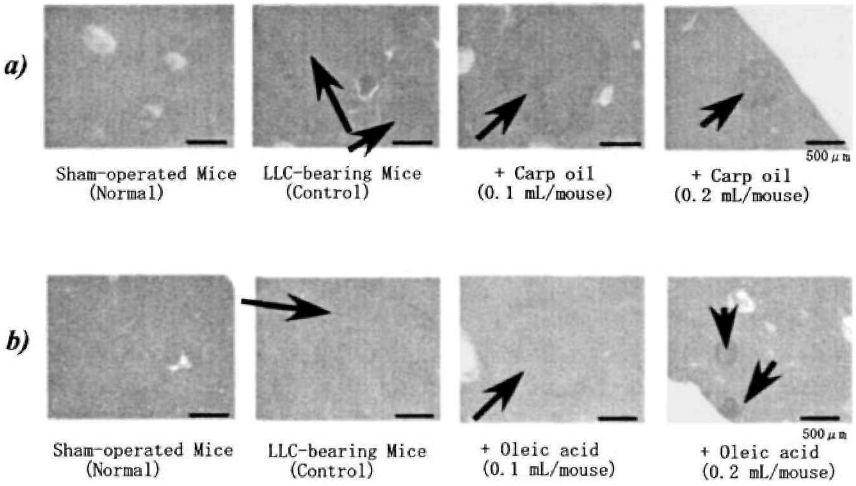



**Fig. (11).** Histology of the inhibition of LLC tumor growth metastasizing in the liver on day 21 by carp oil (a) or oleic acid (b).
Tumors are indicated with arrows.

To characterize the antitumor and/or antimetastatic activities, the effects of carp oil and oleic acid on DNA synthesis in LLC cells, adherence of LLC cells to human adult dermal microvascular endothelial cells (HMVEC), and Matrigel-induced angiogenesis (*in vitro* and *in vivo*) were tested. Carp oil inhibited DNA synthesis in LLC cells at 100 and 1000 μg/mL, but not at 0.1 to 10 μg/mL "Table (**10**)". Oleic acid inhibited DNA synthesis in LLC cells at 1000 μM "Table (**11**)". Carp oil inhibited the Matrigel-induced tube-like network formation of HMVEC at 1000 μg/mL, but not at 0.1 to 100 μg/mL "Table (**10**)".

Oleic acid also significantly inhibited the Matrigel-induced formation of a tube-like network of HMVEC at 10 to 1000 μM, but not at 0.1 or 1 μM "Table (**11**)". Furthermore, carp oil inhibited the adherence of LLC cells to HMVEC at 10 to 1000 μg/mL, but not at 0.1 or 1 μg/mL "Table (**10**)".

On the other hand, oleic acid at 1 or 10 μM slightly increased the adherence of LLC cells to HMVEC "Table (11)".

**Table 10. Effects of carp oil on DNA synthesis in LLC cells, Matrigel-induced capillary-like tube network formation from HMVEC and the adherence of LLC cells to HMVEC (*in vitro*)[1]**

| Carp oil (μg/mL) | [$^3$H]Thymidine incorporation into the DNA of LLC cells (x $10^3$ dpm/well) | Capillary-like tube formation (mm length/field) | Adherence of LLC cells to HMVEC (x$10^3$ LLC cells/well) |
|---|---|---|---|
| 0 | 8.31 ± 1.92 | 58.92 ± 3.06 | 4.19 ± 0.124 |
| 0.1 | 6.33 ± 1.55 | 56.10 ± 4.61 | 4.04 ± 0.165 |
| 1 | 6.11 ± 1.39 | 61.90 ± 5.19 | 3.86 ± 0.131 |
| 10 | 6.48 ± 1.84 | 61.17 ± 5.08 | 3.51 ± 0.099* |
| 100 | 4.61 ± 0.81* | 56.00 ± 3.81 | 2.69 ± 0.142* |
| 1000 | 4.76 ± 0.91* | 32.23 ± 4.83* | 2.43 ± 0.077* |

[1] Values are expressed as means ± S.E. of 4 experiments. * P<0.05, Significantly different from medium alone.

**Table 11. Effects of oleic acid on DNA synthesis in LLC cells, Matrigel-induced capillary-like tube network formation from HMVEC, and the adherence of LLC cells to HMVEC (*in vitro*)[1]**

| Oleic acid (μM) | [$^3$H]Thymidine incorporation into DNA of LLC cells (x $10^3$ dpm/well) | Capillary-like tube formation (mm length/field | Adherence of LLC cells to HMVEC (x$10^3$ LLC cells/well) |
|---|---|---|---|
| 0 | 6.56 ± 0.72 | 63.65 ± 1.83 | 3.78 ± 0.113 |
| 0.1 | 6.34 ± 0.64 | 62.41 ± 1.38 | 3.94 ± 0.104 |
| 1 | 6.29 ± 0.84 | 60.67 ± 0.52 | 5.06 ± 0.453* |
| 10 | 5.58 ± 1.13 | 54.44 ± 1.86* | 4.48 ± 0.182 |
| 100 | 9.82 ± 2.87 | 53.80 ± 1.25* | 4.18 ± 0.098 |
| 1000 | 1.61 ± 0.40* | 48.11 ± 1.45* | 4.09 ± 0.124 |

[1] Values are expressed as means ± S.E. of 4 experiments. * P<0.05, Significantly different from medium alone.

**Table 12. Effects of carp oil on Matrigel-induced angiogenesis (*in vivo*)[1]**

| Treatment | No of animals | Matigel weight (mg) | Hemoglobin (mg/Matrigel) |
|---|---|---|---|
| Matrigel alone | 5 | 77.0 ± 5.82* | 7.0 ± 0.71* |
| Matrigel + VEGF (20 ng/mL) + heparin (32 U/mL) | 5 | 397.2 ± 48.3 | 24.4 ± 4.09 |
| Matrigel/VEGF/heparin | | | |
| + Carp oil (0.1 mL/mouse) | 5 | 405.8 ± 78.2 | 32.0 ± 3.61 |
| (0.2 mL/mouse) | 5 | 163.6 ± 29.3* | 11.8 ± 1.59* |

[1] Values are expressed as means ± S.E. of 5 mice in each group. * P<0.05, Significantly different from Matrigel/VEGF/heparin mixture-treated mice.

As shown in "Tables (**12**) and (**13**)", Matrigel supplemented with 20 ng/mL VEGF, and 32 U/mL heparin, increased the weight of the gel and the hemoglobin concentration in the gels 6 days after implantation, compared with Matrigel alone.
Orally administered carp oil (0.2 mL per mouse) or oleic acid (0.1 or 0.2 mL per mouse) inhibited the increase in the weight and hemoglobin concentration of the gels, induced by the Matrigel/VEGF/heparin mixture "Table (**12**) and Table (**13**)".

**Table 13. Effects of oleic acid on Matrigel-induced angiogenesis (*in vivo*)[1]**

| Treatment | No of animals | Matigel weight (mg) | Hemoglobin (mg/Matrigel) |
|---|---|---|---|
| Matrigel alone | 4 | 74.2 ± 2.12* | 15.0 ± 2.55* |
| Matrigel + VEGF (20 ng/mL) | | | |
| + heparin (32 U/mL) | 4 | 330.4 ± 41.1 | 34.8 ± 5.17 |
| Matrigel/VEGF/heparin | | | |
| + Oleic acid (0.1 mL/mouse) | 4 | 105.8 ± 23.0* | 18.8 ± 1.11* |
| (0.2 mL/mouse) | 4 | 106.6 ± 5.39* | 19.7 ± 0.88* |

[1] Values are expressed as means ± S.E. of 4 mice of each group. * $P<0.05$, Significantly different from Matrigel/VEGF/heparin mixture-treated mice.

These findings suggest that the mechanisms of the antitumor and the antimetastatic actions of carp oil might involve the inhibition of DNA synthesis in LLC cells, and the inhibition of angiogenesis through the inhibition of LLC cell adherence to the microvascular endothelium.
On the other hand, the inhibitory actions of oleic acid on metastasis in the liver and metastatic tumor growth in the liver, cannot be explained by the effects of DNA synthesis in LLC cells, and microvascular endothelial cells, or the adherence of LLC cells to the microvascular endothelium; rather, these inhibitory actions by oleic acid are partly attributable to the inhibition of the angiogenesis induced by tumors. In conclusion, it seems likely that the antitumor and antimetastatic activities of carp oil may be partly ascribed to a fatty acid, oleic acid, as an active substance. However, the antitumor and antimetastatic effects of carp oil are insufficiently by themselves to explain the action of oleic acid. Further work is in progress to identify the active substance(s) in carp oil.

## 2. Vascular Physiological Actions of Natural Products

### a) *Baicalein Isolated from the Roots of Scutellaria baicalensis Inhibits Adhesion Molecule Expression Induced by Thrombin or Thrombin Receptor Agonist Peptide in Cultured HUVECs* [12]

The roots of *Scutellaria baicalensis* Georgi (Labiatae) have been clinically used as therapeutic drugs for the treatment of allergic inflammatory

diseases, such as asthma, atopic dermatitis, hyperlipidemia, and arteriosclerosis, in China and Japan. I reported on the anti-thrombic actions of various flavonoids isolated from Scutellariae Radix (the root of *S. baicalensis*) [13], and the inhibitory action of baicalein (5,6,7-trihydoryflavone), on leukotriene $B_4$ and $C_4$ synthesis, and leukocyte degranulation [14, 15]. The migration of leukocytes into tissue is the central event in the inflammatory response. Leukocyte migration is responsible for the host response to tissue injury and infection, but is also potentially harmful and contributes to the pathology of many diseases and inflammatory disorders. In addition, the migration and invasion of tumor cells into tissue is also a central event in tumor growth and/or metastatic responses. It seems likely that the first steps in this process are the adhesion of leukocytes or tumor cells to the endothelium, and leukocyte or tumor cell transendothelial migration. It has been reported that adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1), were expressed in inflamed tissue in asthma, rheumatoid arthritis and arteriosclerosis, and in primary tumor and metastatic tissue in other organs. Thrombin is a serine protease, which also plays a central role in blood coagulation, platelet activation and pulmonary vascular injury [16-18]. Vu et al. [19] demonstrated that a 14-amino acid peptide (SFLLRNPNDKYEPF), with a sequence identical to the new amino terminus of the thrombin receptor after thrombin cleavage, is able to activate human platelets and directly causes the elevation of the intracellular free calcium concentration ($[Ca^{2+}]i$). In this review, I have introduced the inhibitory actions and the mechanisms of the effects of baicalein, isolated from Scutellariae Radix, on ELAM-1 and ICAM-1 expression, induced by thrombin and thrombin receptor agonist peptide (TRAP), in cultured HUVECs. Thrombin (10 U/mL) and TRAP (160 μM) stimulated ELAM-1 by 2.05- and 2.85-fold after 6 h of incubation, and ELAM-1 expression declined to almost the basal level after 24 h incubation. Thrombin also stimulated ICAM-1 expression after 24 h incubation at concentrations of 5-20 units/mL, dose-dependently, while TRAP did not (data not shown). "Fig. (**12**)" shows the dose-response curve of baicalein on thombin-stimulated ELAM-1 and ICAM-1 expression. Baicalein inhibited the thrombin-stimulated ELAM-1 and ICAM-1 expression dose-dependently; their $IC_{50}$ values were 5.53 ± 1.68 μM for ELAM-1 expression, and 9.67 ± 1.28 μM for ICAM-expression. Baicalein inhibited ELAM-1 expression induced by TRAP with an $IC_{50}$ of 2.44 ± 1.08 μM "Fig. (**13**)". The protein kinase C (PKC) activator, phorbol myristate acetate (PMA), stimulated both ELAM-1 and ICAM-1 expression. The PKC inhibitor H7 (40 μM) and baicalein (50 μM) strongly inhibited PMA-, thrombin- and TRAP-induced ELAM-1 expression. Moreover, PMA- and thrombin-induced ICAM-1 expression were also inhibited by the PKC inhibitor H7 and baicalein "Table (**14**)".

**Table 14. Effects of PKC inhibitor H7 and baicalein on ELAM-1 and ICAM-1 expression induced by PMA, thrombin and TRAP in cultured HUVECs[1]**

| Treatment | % Increase in adhesion molecule expression | |
|---|---|---|
| | ELAM-1 expression[2] | ICAM-1 expression[3] |
| PMA (100 nM) | 249.9±31.4 | 178.9±9.82 |
| + H7[4] (40 μM) | 113.3±34.5* | 73.7±2.77* |
| Thrombin (10 U/mL) | 112.4±23.4 | 381.0±38.7 |
| + H7 (40 μM) | 0.0±0.0* | 0.0±0.0* |
| TRAP (160 μM) | 248.5±29.8 | 34.5±11.6 |
| + H7 (40 μM) | 38.4±10.4* | 47.9±13.9 |
| PMA (100 nM) | 212.4±23.4 | 161.0±8.73 |
| + Baicalein[4] (50 μM) | 80.1±16.3* | 75.4±10.8* |
| TNF-α[5] (5 ng/mL) | 796.7±26.2 | 799.5±32.4 |
| IL-1β[5] (20 ng/mL) | 948.3±102.5 | 516.0±27.2 |

[1] Values are expressed as means ± S.E. of 6-9 experiments. *P<0.01, Significantly different from various stimulators alone. [2] HUVECs were incubated for 6 h at 37°C. [3] HUVECs were incubated for 24 h at 37°C. [4] HUVECs were preincubated with H7 (40 μM) or baicalein (50 μM) for 1 h at 37°C, and then PMA (100 nM), thrombin (10 U/mL) or TRAP (160 μM) was added and the mixture was further incubated for 6 h or 24 at 37°C. [5] TNF-α and IL-1β were used as positive controls.

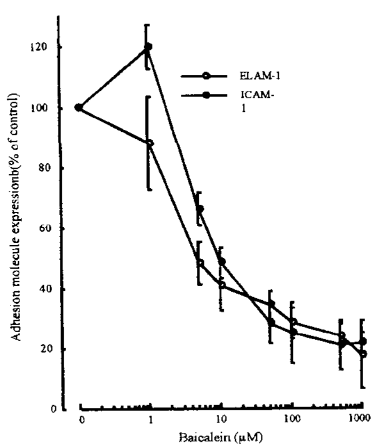



Fig. (12). Effects of baicalein on thrombin-induced ELAM-1 and ICAM-1 expression in cultured HUVECs.
Results are expressed as means ± S.E. of 4-8 experiments. HUVECs were preincubated with the various concentrations of baicalein for 1 h at 37° C, and then thrombin (10 U/mL) was added and the mixture was further incubated for 6 h (ELAM-1 expression) or 24 h (ICAM-1 expression) at 37° C.

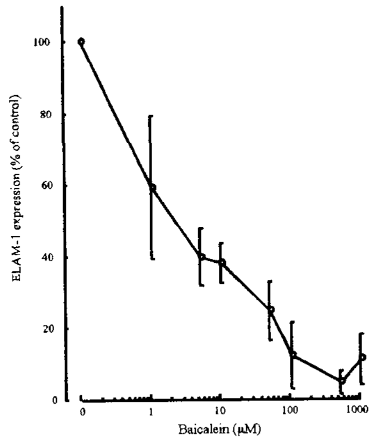



Fig. (13). Effects of baicalein on TRAP-induced ELAM-1 expression in cultured HUVECs.
Results are expressed as means ± S.E. of 4-8 experiments. HUVECs were preincubated with the various concentrations of baicalein for 1 h at 37° C, and then TRAP (160 mM) was added and the mixture was further incubated for 6 h at 37° C.

These findings suggest that the inhibitory effects of baicalein on thrombin-induced ELAM-1 and ICAM-1 expression, or TRAP-induced ELAM-1 expression, may be due to the inhibition of PKC activation. I previously reported that baicalein had potent anti-allergic and

anti-inflammatory reactions. Therefore, the anti-allergic and anti-inflammatory actions of baicalein can partly be explained by the inhibitory effects on adhesion molecule expression in vascular endothelial cells.

*b) Artery relaxation by chalcones isolated from the roots of Angelica keiskei and the mechanisms involved* [20]

*Angelica keiskei* Koidzumi ("Ashitaba" in Japanese) is a plant, found along the Pacific Coast of Japan, that is used as a diuretic, laxative, analeptic and lactagogue. Although it has recently been thought that the roots and herbs of *A. keiskei* have preventive effects on coronary heart diseases and hypertension, the basis for this hearsay is unclear. Therefore, in the preliminary experiments to clarify whether the roots and herbs have preventive effects on coronary heart disease and hypertension, the effects of a 50% ethanol extract on catecholamine-induced vasoconstriction of the rat aorta were examined. I found that the 50% ethanol extract (500 μg/mL) of the roots of *A. keiskei* inhibited catecholamine-induced vasoconstriction of the rat aorta "Table (**15**)". Therefore, I isolated and identified the active substances using a phenylephrine-induced vasoconstrictive assay to guide fractionation. The EtOAc-soluble fraction obtained from the 50% ethanol extract inhibited phenylephrine-induced vasoconstriction at a concentration of 100 μg/mL, but the EtOAc-insoluble fraction had no effect at the same concentration. The EtOAc fraction was chromatographed on silica gel eluted with increasing concentrations of EtOAc in n-hexane "Table (**15**)".

**Table 15. Effects of various fractions extracted from the roots of *Angelica keiskei* of phenylephrine-induced vasoconstriction in rat aortic rings[1]**

| Treatment | | Phenylephrine-induced contraction (% of control) |
|---|---|---|
| Phenylephrine (0.1 μM) | | 100 ± 7.94 |
| + 50% EtOH extract | | |
| | (100 μg/mL) | 82.4 ± 3.01* |
| | (250 μg/mL) | 70.2 ± 1.72* |
| | (500 μg/mL) | 49.3 ± 1.45 |
| + EtOAc-soluble fraction | | |
| | (50 μg/mL) | 79.1 ± 0.89* |
| | (100 μg/mL) | 71.0 ± 1.55* |
| | (200 μg/mL) | 52.6 ± 1.57* |
| + EtOAc-insoluble fraction | | |
| | (50 μg/mL) | 105.3 ± 2.51 |
| | (100 μg/mL) | 111.1 ± 3.16 |
| | (200 μg/mL) | 88.9 ± 2.11 |
| + Carbachol (3 μM)[2] | | 2.70 ± 0.71* |

[1] Values are expressed as means ± S.E. of three experiments. [2] Positive control. *P<0.05, Significantly different from phenylephrine alone.

The 20% EtOAc eluate inhibited phenylephrine-induced vasoconstriction and was further rechromatographed on silica gel eluted with $CHCl_3$-MeOH (100: 1 to 30: 1), and Lichrorep RP-18 Lobar column with 80% MeOH, to yield the active substances (compounds 1-5) that inhibit phenyleohrine-induced vasoconstriction.

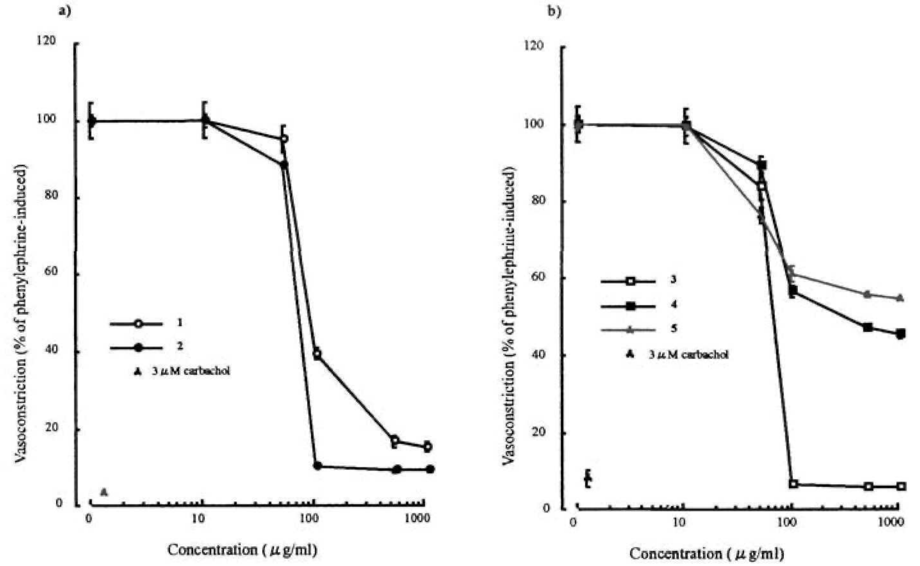



**Fig. (14).** Effects of compounds 1 and 2 (a), and compounds 3-5 (b), isolated from the roots of *A. keiskei* on phenylephrine-induced vasoconstriction in rat aortic rings.
Results are expressed as means ± S.E. of three experiments. The degree of phenylephrine-induced vasoconstriction is expressed as 100%.

As shown in "Fig. (**14**)", compounds 1-5 inhibited phenylephrine-induced vasoconstriction. Compounds 1, 2, 3, 4 and 5 were identified as xanthoangelol, 4-hydroxyderricin, and xanthoangelols B, E and F, respectively, based on the comparison of their spectral data with those of authentic samples. The structures of the five isolated active substances are shown in "Fig. (**15**)".

The degree of inhibition of phenylephrine-induced vasoconstriction was in the order xanthoangelol B> 4-hydroxyderricin> xanthonagelol> xanthoangelol E = xanthoangelol F, at a concentration of 100 μg/mL. As shown in "Fig. (**16**)", 4-hyddroxyderricin inhibited the phenylephrine-induced vasoconstriction of the intact aortic ring with the endothelium, while the inhibitory action of 4-hydroxyderricin on phenylephrine-induced vasoconstriction was attenuated by the destruction of the endothelium with Triton X-100 "Fig. (**16**)", or by adding $N^G$-monomethyl-L-arginine (L-NMMA; a nitric oxide (NO) synthetase

inhibitor) (data not shown). Xanthoangelol, xanthoangelol E, and xanthoanbgelol F, also caused less inhibition of phenylephrine-induced vasoconstriction in the presence of the NO synthetase inhibitor L-NMMA, and in aortic rings in which the endothelium had been destroyed "Fig. (**16**)". Among the five chalcones, it was shown that the strongest inhibition of phenylephrine-induced vasoconstriction was from xanthoangelol B. Xanthoangelol B inhibited the phenylephrine-induced vasoconstriction of the aortic ring, with or without the endothelium "Fig. (**16**)", and with or without L-NMMA (data not shown).

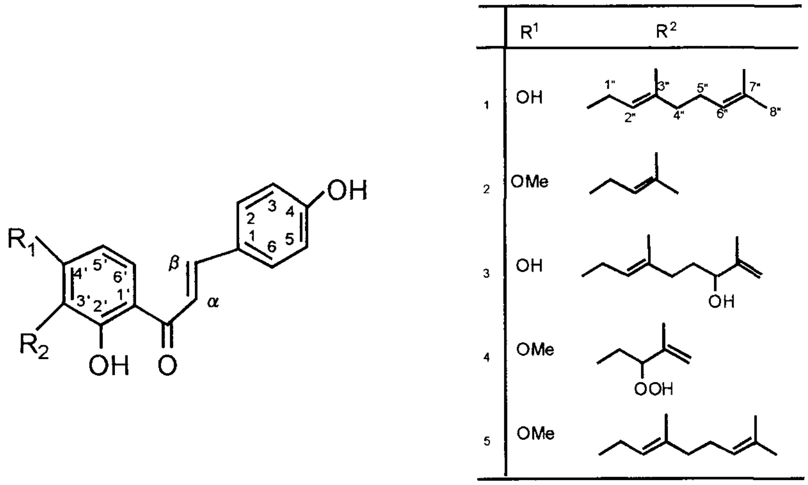



**Fig. (15).** Structures of compounds 1-5.
Compound 1, xanthoangelol; compound 2, 4-hydroxyderricin; compound 3, xanthoangelol B, compound 4, xanthoangelol E; compound 5, xanthoangelol F.

As shown in "Fig. (**17**)", 4-hyddreoxyderricin, at a concentration of 10 or 50 μg/mL, inhibited the elevation of [$Ca^{2+}$]i levels induced by phenylephrine in cultured rabbit aortic smooth muscle cells. Xanthoangelol B at 50 or 100 μg/mL also inhibited the phenylephrine-induced [$Ca^{2+}$]i elevations in cultured rabbit smooth muscle cells "Fig. (**18**)".

From these results, it seems likely that the inhibitory mechanism of 4-hydroxyderricin on catecholamine-induced vasoconstriction might be mediated through the reduction of catecholamine-induced [$Ca^{2+}$]i elevation, and/or by affecting the production of the endothelium-derived relaxing factor (EDFR)/NO in the endothelium. Xanthoangelol and xanthoangelols E and F also appeared to inhibit catecholamine-induced vasoconstriction through endothelium-dependent production of EDRF/NO. On the other hand, the inhibitory mechanism of xanthoangelol B on

catecholamine-induced vasoconstriction might involve the direct inhibition of smooth muscle function without affecting EDFR/NO production in the endothelium.

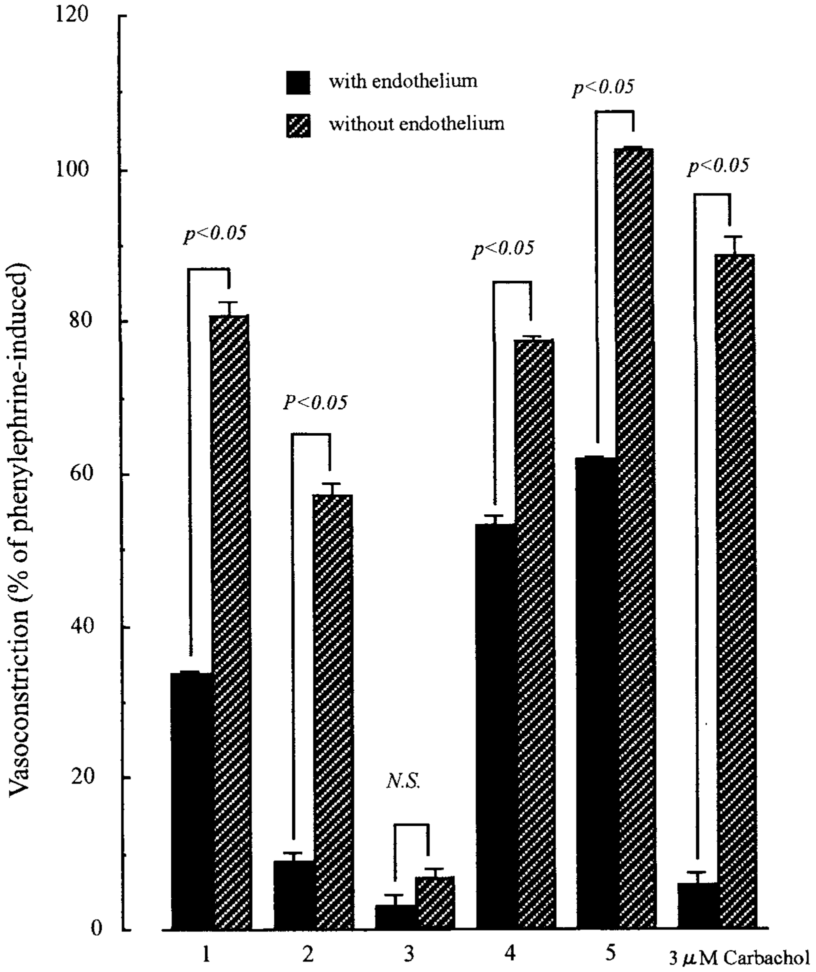



**Fig. (16).** Effects of five chalcones isolated from the roots of *A. keiskei* on phenylephrine-induced vasoconstriction with or without the endothelium.
Results are expressed as means ± S.E. of three expreriments.
The degree of 0.1 μM phenylephrine-induced vasoconstriction is expressed as 100%.
Concentration of each tested compound: 100 μg/mL.
1, xanthoangelol; 2, 4-hydroxyderricin; 3, xanthoangelol B; 4, xanthoangelol E;
5, xanthoangelol F; Positive control, 3 μM carbachol.

Examination of the chemical structures of the five chalcones revealed that

they all contain 3'-substituted 2',4-dihydroxy-4'-hydroxy- or –methoxychalcone. The side chain at the C-3' position of xanthoangelol and xanthoangelol F is a geranyl [(*E*)-3,7-dimehyl-2,6-octadienyl] moiety, and it was found that these compounds inhibited catecholamine-induced vasoconstriction through endothelium-dependent NO/EDFR production. The inhibitory action of 4-hydroxyderricin, which has a γ, γ-dimethylallyl group at the C-3' position, was stronger than that of xanthoangelol or xanthoangelol F, which have a geranyl group at this position. Furthermore, the inhibitory action of xanthoangelol E, in which a 2-hydroperoxy-3-methyl-3- butenyl group is substituted for a γ, γ-dimethylallyl (3-methyl-2-butanyl) group of 4-hydorxyderricin at the C-3'position, was attenuated in comparison to that of 4-hydsroxyderricin.

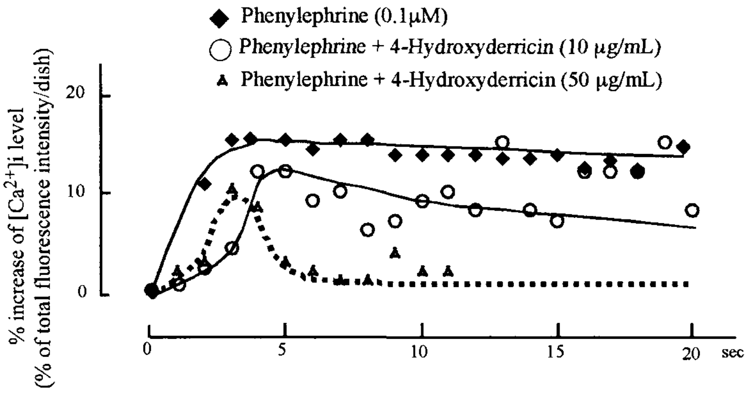



**Fig. (17).** Effects of 4-hydroxyderricin on $[Ca^{2+}]i$ levels in cultured rabbit aortic smooth muscle cells treated with phenylephrine.

Fluo 3-loaded rabbit aorta smooth muscle cells were preincubated for 2 min with DMEM medium (phenol red free) containing 10% fetal bovine serum or 4-hydroxyderricin at 37°C, and then phenylephrine (0.1 mM) was added and the measurement of $[Ca^{2+}]i$ was monitored by a Laser Cytometer (ACAS 570, MEPIDIAN, USA) with excitation at 488 nm and emissions at 530 nm. The total $[Ca^{2+}]i$ concentration per dish of rabbit aorta smooth muscle cells was determined by adding 0.1% Triton X-100, and $[Ca^{2+}]i$ values were expressed as % of increase of $[Ca^{2+}]i$ level.

The inhibitory actions of 4-hydroxyderricin and xanthoangelol E were also shown to be mediated through endothelium-dependent NO/EDFR production. These findings suggest that the difference in the side chain at the C-3' position of these chalcones may be closely related to their inhibitory strength. On the other hand, it was of great interest that xanthoangelol B, with an (*E*)-6-hydroxy-3,7-dimethyl-2,7-ocatdienyl moiety at the C-3', position inhibited catecholamine-induced vasoconstriction without affecting endothelium-dependent NO/EDFR

production. The difference between the inhibitory mechanisms of xanthoangelol B, with an (*E*)-6-hydroxy-3,7-dimethyl-2,7-octadienyl moiety, and xanthoangelol or xanthoangelol F, with an (*E*)-3,7-dimethyl-2,6-octadienyl moiety, may be due to the difference in their side chains at the C-3' position. Further experiments examining a number of related compounds are needed to test this hypothesis.

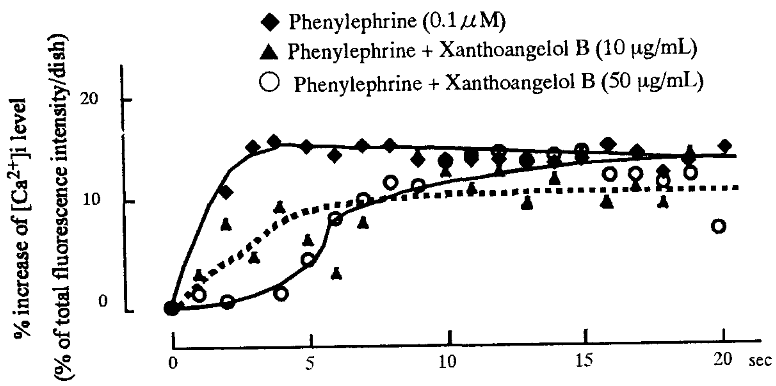



**Fig. (18).** Effects of xanthoangelol B on $[Ca^{2+}]i$ levels in cultured rabbit aortic smooth muscle cells treated with phenylephrine.

Fluo 3-loaded rabbit aorta smooth muscle cells were preincubated for 2 min with DMEM medium (phenol red free) containing 10% fetal bovine serum or xanthoangelol B at 37°C, and then phenylephrine (0.1 mM) was added and the measurement of $[Ca^{2+}]i$ was monitored by a Laser Cytometer.

## REFERENCES

[1] Kimura, Y.; Okuda, H. In *Studies in Natural Products Chemistry*; ATT-ur-Rahaman, Ed.; Elsevier Science B.V: Amsterdam, **2002**; Vol.*27*, in print.

[2] Takaku, T.; Kimura, Y. ; *Jap. J. Constitutional Med*, **2002**, *64*, 80-85.

[3] Kimura, Y.; Taniguchi, M.; Baba, K.; *Anticancer Res*, **2002**, in print.

[4] Kimura, Y; Okuda, T.; Arichi, S.; *Planta Medica*, **1988**, *54*, 290-293.

[5] Miyazaki, T.; Nishijima, M.; *Chem. Pharm. Bull*; **1981**, *29*, 3611-3616.

[6] Maruyama, H.; Yamazaki, K.; Murofushiu, S.; Konda, C.; Ikekawa, T.; *J. Pharmacobiodyn* **1989**, 12, 118-123.

[7] Wang, G.; Zhang, J.; Mizuno, T.; Zhuang, C.; Ito, H.; Mayuzumi, H.; Okamoto, H.; Li, J.; *Biosci. Biotechnol. Biochem*, **1993**, *57*, 894-900.

[8] Wang, S.Y.; Hsu, H.C.; Tzeng, C.H.; Lee, S.S.; Shiao, M.S.; Ho, C.K.; *Int. J. Cancer*, **1997**, *70*, 699-705.

[9] Shang, L.; Zhang, M.; Zhou, Q.: Chen, J.; Zeng, F.; *Biosci. Biotechonol. Biochem*, **2000**, *64*, 2172-2178.

[10] Min, B-S.; Cao, J-J.; Nakamura, N.; Hattori, M.; *Chem. Pharm. Bull*, **2000**, 48, 1026-1033.

[11] Kimura, Y.; *J. Nutr*, **2002**, *132*, 2069-2075.

[12] Kimura, Y.; Matsushita, N.; Yokoi-Hayashi, K.; Okuda, H.; *Planta Medica*, **2001**, *67*, 331-334.

[13] Kubo, M.; Mstsuda, H.; Tani, T.; Arichi, S.; Kimura, Y.; Okuda, H.; *Chem. Pharm. Bull*, **1985**, *33*, 2411-2415.

[14] Kimura, Y.; Okuda, H.; Arichi, S.; *Phytotherapy Res*, **1987**, *1*, 48-49.

[15] Kimura, Y.; Okuda, H.; Arichi, S.; *Biochim. Biophys. Acta*, **1987**, *922*, 278-286.

[16] Furie, B.; Furie, B.C.; *Cell*, **1988**, *53*, 505-518.

[17] Kimura, Y.: Okuda, H.; Arichi, S.; *J. Pharm. Pharmacol*, **1988**, *40*, 838-843.

[18] Tahamont M.V.; Malik, A.B.; *J. Appl. Physiol. Respir. Environ. Exercise Physiol*, **1983**, *54*, 1489-1495.

[19] Vu, T.K.H.; Wheaton, V.I.; Hung, D.T.; Charo, I.; Coughlin, S.R.; *Cell*, **1991**, *64*, 1057-1068.

[20] Matsuura, M.; Kimura, Y.; Nakata, K.; Baba, K.; Okuda, H.; *Planta Medica*, **2001**, *67*, 230-235.

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# ANTI-OBESITY EFFECTS OF NATURAL PRODUCTS

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**ABSTRACT:** Natural products such as tea saponin, *Platycodi radix* saponin, chitosan and chondroitin sulfate were found to inhibit the hydrolysis of triolein emulsified with phosphatidylcholine by pancreatic lipase in vitro. These products reduced the elevation of the rat plasma triacylglycerol level after an oral administration of a lipid emulsion containing corn oil. Based on these results, experiments were designed to test whether these products prevented obesity induced by feeding a high fat diet to mice. All these natural products were proved to prevent the increase in parametrial adipose tissue weight of mice fed the high fat diet. Such anti-obesity action of these products may be mediated through delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity.

## INTRODUCTION

It is well known that dietary fat is not absorbed from the intestine unless it has been subjected to the action of pancreatic lipase [1]. Previously, we found that basic proteins such as protamines, histones and purothionine inhibited the hydrolysis of triolein emulsified with phosphatidylcholine [2]. The inhibition of hydrolysis of dietary fat may cause a decrease or delay in the intestinal absorption of fat and reduce blood chylomicron levels, an excess of which is known to induce obesity [3]. Therefore, there was a possibility that inhibitory substances toward pancreatic lipase activity may prevent the onset of obesity induced by feeding a high fat diet to mice. Recently, we found that natural products such as tea saponin, platycodi radix saponin, chitin-chitosan and chondroitin sulfate inhibited the pancreatic lipase activity. In the following section, the anti-obesity effects of these natural products will be described in detail.

**Tea saponin**

Three kinds of tea, oolong, green and black, have been widely used as health drinks from ancient times all over the world, especially to prevent obesity and improve lipid metabolism. Among the three teas, oolong tea is traditionally reported to have anti-obesity and hypolipidemic actions. Kimura et al. [4] reported that the three kinds of tea prevented the elevations of serum and liver lipids (total cholesterol and triglyceride) and liver injury with an increase in serum transaminases (glutamic pyruvic transaminase and glutamic oxaloacetic transaminase) in rats fed peroxidized oil for one week. Furthermore, we found that tea tannins such as epigallocatechin, epicatechin gallate and epigallocatechin gallate, strongly inhibited lipid peroxidation in rat liver mitochondria and microsomes [5]. Recently, we found that obesity was induced by feeding a high-fat diet containing 40% beef tallow for 10 weeks to female mice [6]. In the present study, we used a high-fat diet-induced model of obesity in mice to clarify whether oolong tea prevents obesity. In addition, we attempted to isolate the anti-obesity effectors from oolong tea using an inhibitor assay on pancreatic lipase.

Oolong tea produced from Fu-Jian Sheng in China was purchased from Uchida Wakan-Yak Ind. (Osaka, Japan), and voucher samples are stored at the 2nd Department of Medical Biochemistry, School of Medicine, Ehime University. The botanical classification of oolong tea was identified as *Thea sinensis* L. by Prof. Dr. M. Kubo (Department of Natural Drug Resources, Faculty of Pharmaceutical Sciences, Kinki University, Japan) and Prof. Dr. Y. Zheng (Chinese Medicine Material College, Jilin Agricultural University, China). Voucher specimens of oolong tea were matched with specimens in the botanical repository of the above two universities in Japan and China.

Female ICR mice (three weeks old) were obtained from CLEA (Osaka, Japan), and maintained under a 12h/12h light/dark cycle, in a temperature- and humidity-controlled room. The animals were given laboratory pellet chow (CLEA Japan Inc; protein 24%, lipid 3.5%, carbohydrate 60.5%) and water *ad libitum*. Fifty-four mice were divided into three groups (n=18 each), with the groups matched for body weight, after one week of feeding. The control mice were fed laboratory pellet chow (normal group). The mice in the high-fat diet-fed groups received the high-fat diet (beef tallow 40%, casein 36%, corn starch 10%, sugar 9%, vitamin mixture 1% and mineral mixture 4% w/w per 100 g diet)

and water for 10 weeks *ad libitum*. The mice in the experimental group received the high-fat diet containing 5% oolong tea powder (raw plant dry leaf) for 10 weeks. The body weight of each mouse was estimated once a week. The total amount of food intake by each mouse was recorded at least three times every week. Following overnight starvation they were sacrificed after anaesthetization with diethyl ether for about 2 min, and the liver and parametrial white adipose tissues were quickly removed. The liver tissues were stored at – 80°C until analysis was performed. The liver triglyceride content was estimated as follows; a portion (0.5 g) of the liver tissue was homogenized in Krebs Ringer phosphate buffer (pH 7.4, 4.5 ml), and the homogenate (0.2 ml) was extracted with chloroform-methanol (2:1, v/v, 4 ml). The extract was concentrated under a nitrogen stream, and the residue was analyzed using a Triglyceride E-Test kit.

Fig. (**1**) shows the changes in body weights of the g roups during the experiment. Feeding a high-fat diet containing 40% beef tallow for 10 weeks produced significant increases in body weight and parametrial white adipose tissue weight compared to laboratory chow-fed mice. Furthermore, the high-fat diet also induced fatty liver, with the accumulation of triglyceride when compared to the normal group (Table 1). Feeding a high-fat diet containing 5% oolong tea powder reduced the increases in body weight and final parametrial adipose tissue weight, and the accumulation of liver triglyceride compared to the high-fat diet group (Fig. (**1**) and Table 1).

**Table 1** Effects of oolong tea on parametrial adipose tissue weight, liver weight and hepatic triyglyceride in mice fed a high fat diet for 10 weeks.

| Group | Mean | | |
|---|---|---|---|
| | **Parametrial adipose tissue (g)** | **Liver (g)** | **Hepatic triglyceride (mg/g)** |
| Lean control group | 0.89 | 1.27 | 63.3 |
| High-fat diet-treated group | 1.39 | 2.24 | 116.5 |
| High-fat diet plus 5% oolong tea powder-group | 0.67 | 2.12 | 49.8 |

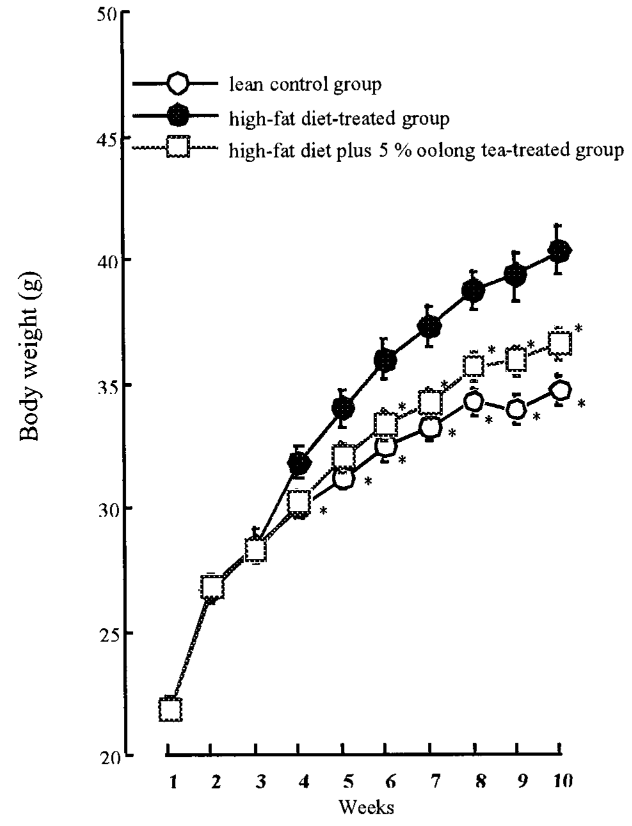



**Fig. (1).** Effects of oolong tea on body weight in mice fed a high-fat diet for 10 weeks. Values are expressed as mean ± s.e.m. of 18 mice in each group. Significantly different from the high-fat diet-treated group; *$p<0.05$.

The rate of reduction in body weight corresponded with that in parametrial adipose tissue weight. The mean food consumption per week per mouse during the whole experimental period was significantly different between the laboratory chow and high-fat diet groups, being 98.5±2.13 kcal in the laboratory chow group and 147.8±7.8 kcal in the high-fat diet group. There was no significant difference in food consumption between the high-fat diet group and high-fat plus oolong tea diet (161.5±8.9 kcal per mouse per week). These results suggested that the prevention of high-fat diet-induced obesity by oolong tea may be due to a decrease in the intestinal absorption of lipid. Then, we attempted to isolate an anti-obesity effector from oolong tea using an inhibitory assay for pancreatic lipase. It has been clinically reported that

a pancreatic lipase inhibitor orlistat (Ro 18-0647) prevented obesity and hyperlipidaemia after treatment for 12 weeks through the inhibition of fat absorption [7-10].

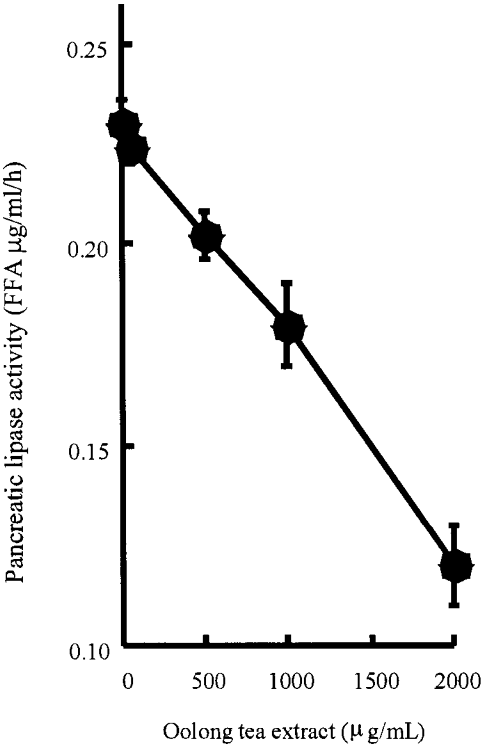



**Fig. (2).** Effects of the water extract of oolong tea on pancreatic lipase activity. Values are expressed as mean ± s.e.m. of four experiments. FFA=free fatty acid.

In the present study, we found that the water extract of oolong tea inhibited pancreatic lipase at the concentrations of 0.5-2 mg/ml, in a dose-dependent manner, Fig. (**2**). Although caffeine was identified as an activator of noradrenaline-induced lipolysis, it failed to inhibit the pancreatic lipase activity (data not shown). Tea tannins such as epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate also had no effect on the pancreatic lipase activity (data not shown). These results suggest that the inhibition of pancreatic lipase activity may be caused by tea saponin fractions in oolong tea that differ from caffeine and tannins. In this experiment, we found that tea saponin (a mixture of theasaponins $E_1$ and $E_2$) isolated from oolong tea inhibited

pancreatic lipase *in vitro*, Fig. (**3**). Furthermore, an experiment was designed to clarify whether or not tea saponin prevented obesity induced by long-term feeding of a high-fat diet containing 40% beef tallow. Female ICR mice (three weeks old) were divided into three groups, with each group matched for body weight, after one week of being fed laboratory pellet chow *ad libitum*. The control group (n=10) continued to be fed laboratory pellet chow *ad libitum*. The high-fat diet group (n=14) was given the high-fat diet containing 40% beef tallow, 36% casein, 10% corn starch, 9% sugar, 1% vitamin mixture and 4% mineral mixture. The high-fat diet plus tea saponin group (n=12) was given the high-fat diet containing 40% beef tallow, 35.5% casein, 10% corn starch, 9% sugar, 1% vitamin mixture and 4% mineral mixture with tea saponin mixed in at a concentration of 0.5%. The mean food consumption per week per mouse was significantly different between the control group and high-fat diet groups, being 430±12 kJ in the control group and 487±17 kJ in the high-fat diet group, but not significantly different between the high-fat and high-fat plus 0.5% tea saponin diet groups, being 487±17 kJ (high-fat diet) and 582±59 kJ (high-fat plus 0.5% tea saponin). Feeding a high-fat diet plus tea saponin had no effect on stool frequency and content, but significantly increased the triacylglycerol contents in feces compared to feeding a high-fat diet (triacylglycerol contents in feces: control group, 4.3±0.2; high-fat diet group, 37.2±6.7; high-fat plus 0.5% tea saponin diet group, 81.9±26.4 mmol/g). Fig. (**4**) shows the changes in body weights of the groups during the experiments. Feeding a high-fat diet for 11 weeks caused significant increases in body weights at 5 to 11 weeks compared to the control group (laboratory pellet chow). Feeding a high-fat diet with 0.5% tea saponin significantly suppressed the increase in body weight compared to the high-fat diet during the treatment period.

**Table 2.** Effects of tea saponin on parametrial adipose weight and the diameter of adipose cells of mice fed a high-fat diet for 11 weeks.

| Group | Mean | |
|---|---|---|
| | **Parametrial adipose (g)** | **Cell diameter ( μ m)** |
| Control | 0.98 | 90.4 |
| High-fat diet | 2.08 | 117.6 |
| High-fat diet+0.5% teasaponin | 1.04 | 83.5 |

The final parametrial adipose tissue weights of the groups are shown in Table 2. The final parametrial adipose tissue weight was significantly increased by feeding a high-fat diet compared to the control group. That in animals with a high-fat diet containing 0.5% tea saponin was significantly reduced compared to the high-fat diet group. As shown in Table 2, the diameter of fat cells was significantly greater in the high-fat diet group than in the control group, and tea saponin completely prevented the high-fat diet-induced increase in cell diameter.

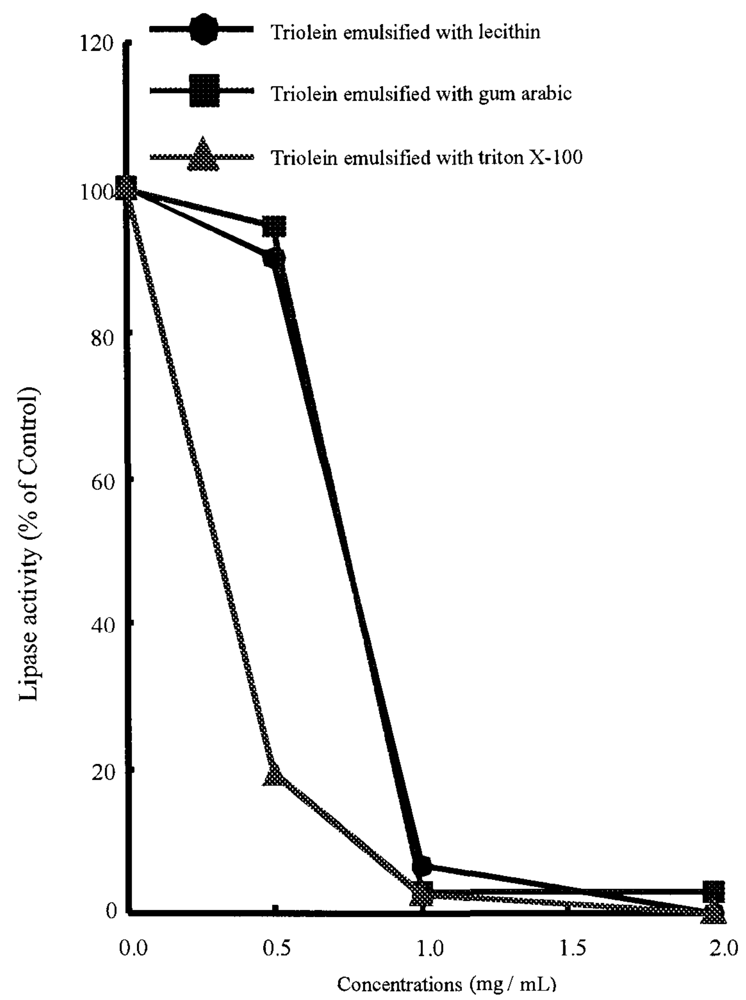



**Fig. (3)** Effects of tea saponin on pancreatic lipase activity. Results are expressed as means ± s.e.m. of four experiments.

It was demonstrated that the anti-obesity effects of oolong tea in high-fat diet-treated mice might be partly due to the inhibitory actions of the saponin fraction in oolong tea on pancreatic lipase activity. In this study, tea saponin prevented the high-fat diet-induced increases in body and parametrial adipose tissue weights by inhibiting the intestinal absorption of dietary fat via inhibition of pancreatic lipase activity, Fig. (5).

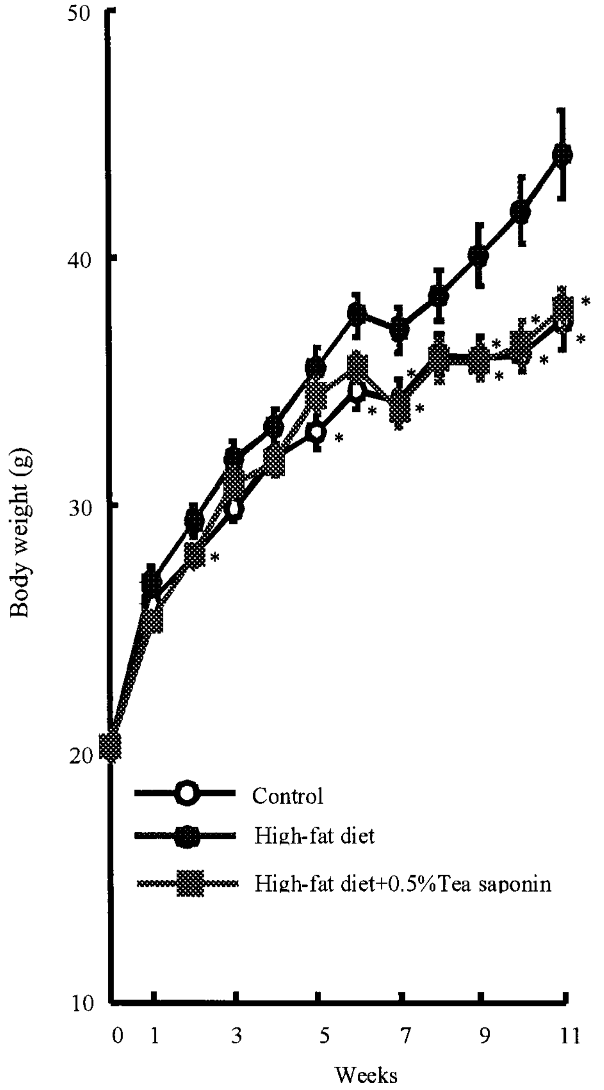



**Fig. (4)** Effects tea saponin on body weight in mice fed a high-fat diet for 11 weeks. Results are expressed as mean ± s.e.m. of 10-14. *p<0.05, Significantly different from high-fat diet group.

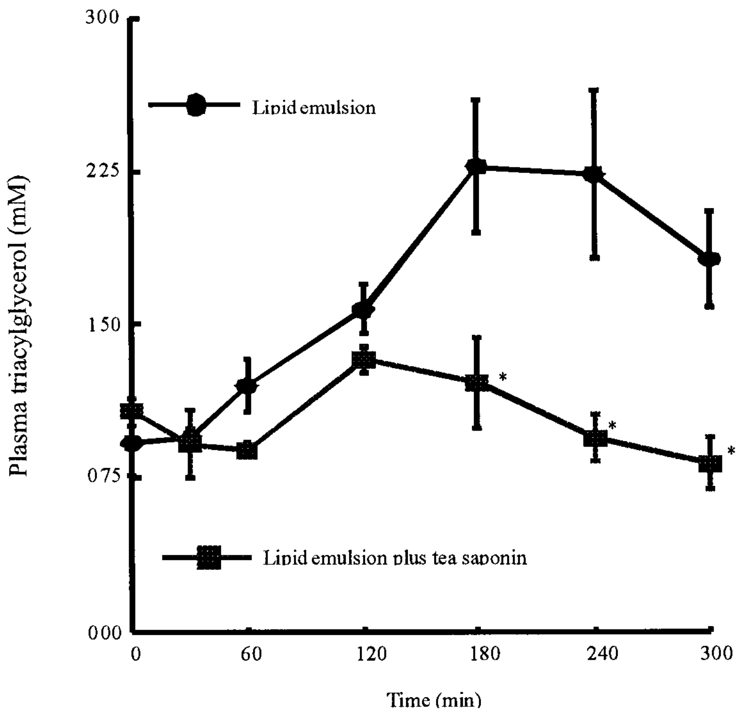



**Fig. (5)** Effects of tea saponin on rat plasma triacylglycerol levels after oral administration of a lipid emulsion. Each point represents the mean ±s.e.m. of four rats. *p<0.05, significantly different from lipid emulsion only-treated group.

## Platycodi radix saponin

Platycodi radix, the roots of Platycodon grandiflorum (Jacq.) A.DC., has been used traditionally as an expectorant and a remedy for bronchits, tonsillitis, laryngitis and suppurative dermatitis in China, Korea and Japan. In China and Korea, the fresh roots of P. grandiflorum have been eaten as pickles for preventing obesity. Although it has been thought recently that Platycodi radix pickles have anti-obesity activity, only hearsay evidence exists. It has been reported that Platycodi radix prevented hypercholesterolaemia and hyperlipidaemia [11]. However, the mechanism of the anti-obesity and -hyperlipidaemic effects of Platycodi radix remain to be clarified. In preliminary experiments, we examined the effects of the aqueous extract of Platycodi radix on pancreatic lipase activity in vitro and on the elevation of plasma triacylglycerol levels caused by the oral administration of a lipid emulsion to rats.

The aqueous extract of Platycodi radix inhibited the pancreatic lipase activity in a dose-dependent manner in the assay system using triolein emulsified with phosphatidylcholine, Fig. (**6**). At 2, 3 and 4 h after the administration of the aqueous extract of Platycodi radix, the plasma triacylglycerol concentration was significantly lower in the treated rats than in the control group, Fig. (7).

**Table 3.** Effects of the aqueous extract of Platycodi radix on liver weight, liver triacylglycerol and total cholesterol in mice fed the high-fat diet for 8 weeks.

| Group | Mean | | |
|---|---|---|---|
| | **Liver weight (g/100g body weight)** | **Triacylglycerol (μ mol/g liver)** | **Total cholesterol (μ mol/g liver)** |
| High-fat diet | 7.21 | 142.49 | 13.6 |
| High-fat plus 2% aqueous extract of Platycodi radix | 6.35 | 118.43 | 13.8 |
| High-fat plus 5% aqueous extract Platycodi radix | 6.17 | 109.02 | 14.1 |

On the basis of these results, the principal study was designed to clarify whether the aqueous extract of Platycodi radix prevented obesity induced by feeding a high-fat diet for 8 weeks. Energy consumption at 4-8 weeks was not significantly different among the high-fat diet and the high-fat diet plus aqueous extract of Platycodi radix (2 or 5%) group as follows: 753.0±33.0 kJ/week/mouse in the high-fat diet group; 712.7±35.2 kJ/week/mouse in the high-fat diet plus 2% aqueous extract of Platycodi radix group; 682.8±45.8 kJ/week/mouse in the high-fat diet

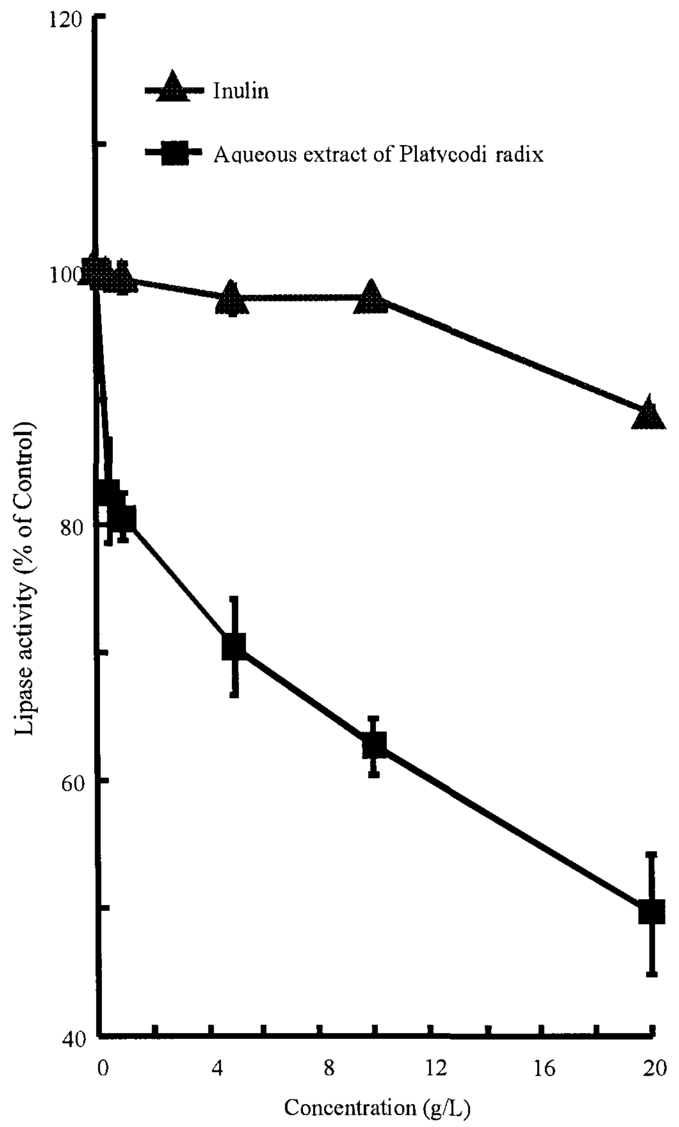



**Fig.** (6) Effects of the aqueous extract of Platycodi radix and inulin isolated from the aqueous extract of Platycodi radix on pancreatic lipase activity *in vitro*. Results are expressed as mean ± s.e.m. of four experiments.

plus 5% aqueous extract of Platycodi radix group. Consumption of the high-fat diet for 8 weeks caused significant increases in body, parametrial

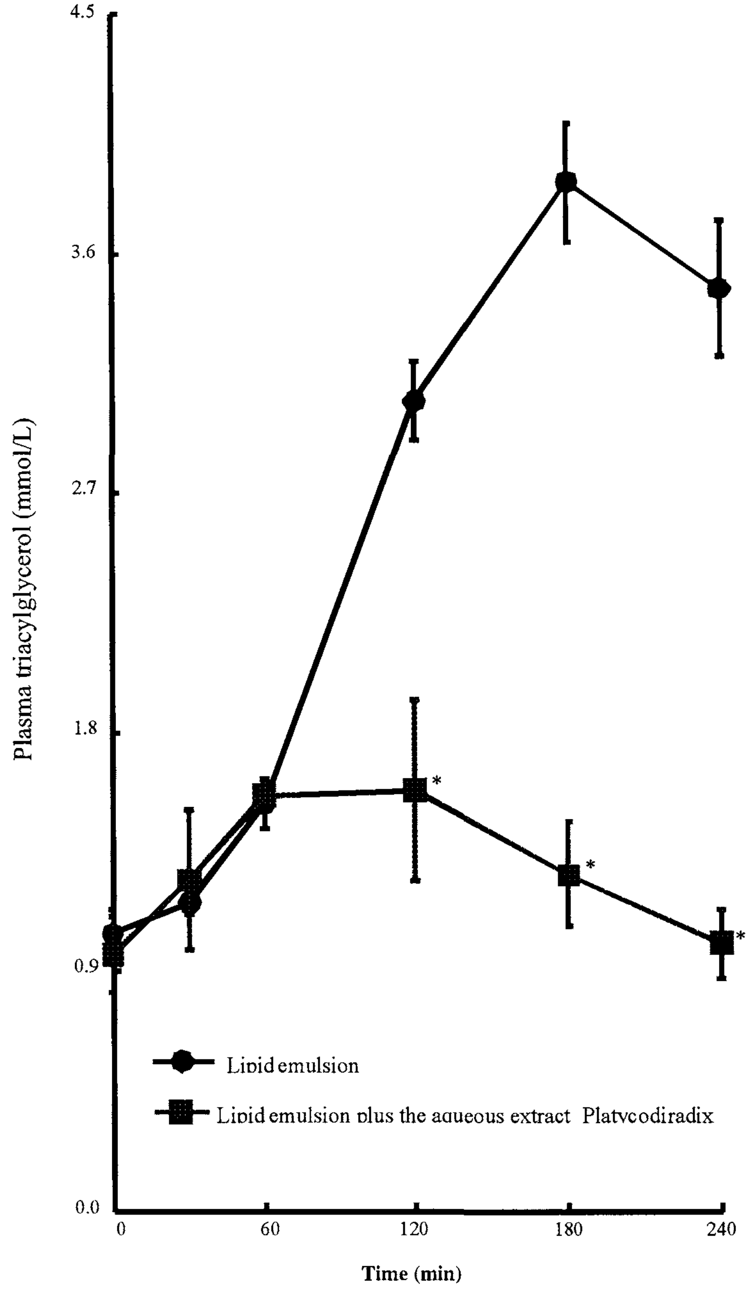



**Fig. (7)** Effect of the aqueous extract of Platycodi radix on rat plasma triacylglycerol level after oral administration of a lipid emulsion. Each point represents the mean ± s.e.m. of three experiments. *$p<0.05$, significantly different from the group treated with lipid emulsion alone at the same time.

adipose tissue and liver weights. Body weight at 3 to 8 weeks, Fig. (8) and final parametrial adipose tissue, Fig. (9) and liver

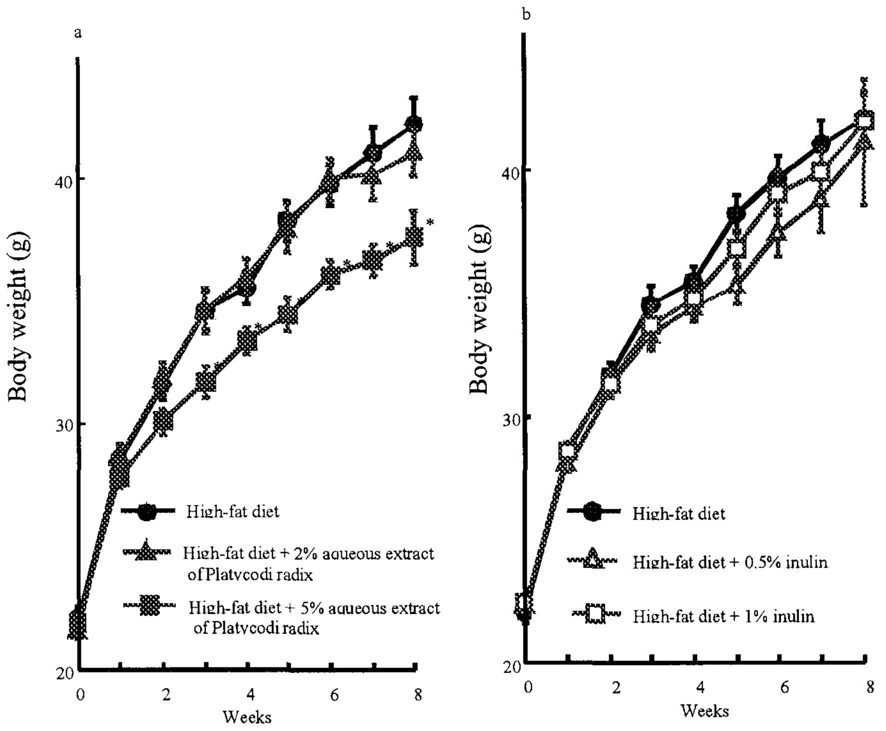



**Fig. (8)** Effects of the aqueous extract of Platycodi radix (A) and inulin isolated from the aqueous extract of Platycodi radix (B) on body weight in mice fed a high-fat diet for 8 weeks. Results are expressed as means ± s.e.m., n=14. *$p<0.05$, significantly different from high-fat diet goup.

weights (Table 3) were significantly reduced by consumption of the high-fat diet containing 5% aqueous extract of Platycodi radix compared with feeding the high-fat diet alone. Feeding the high-fat diet containing 2% aqueous extract of Platycodi radix did not reduce the body or parametrial adipose tissue weights. On the other hand, feeding the high-fat diet containing 2% aqueous extract of Platycodi radix significantly reduced liver weight. Feeding the high-fat diet caused fatty liver with accumulation of triacylglycerol and total cholesterol (Table 3).

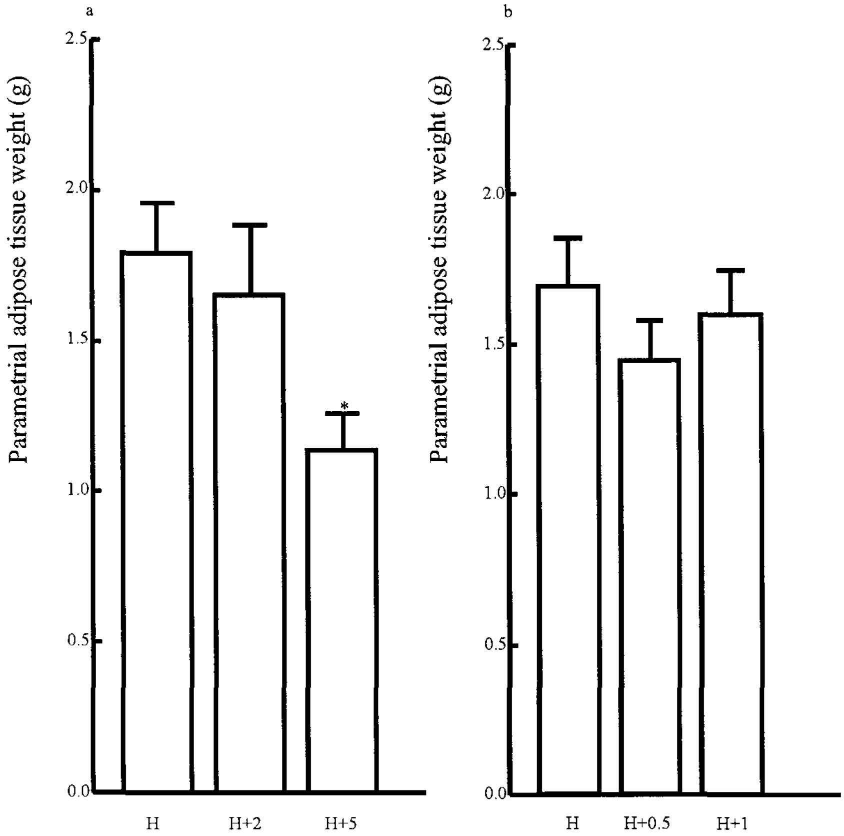



**Fig. (9)** Effects of the aqueous extract of Platycodi radix (A) and inulin isolated from the aqueous extract of Platycodi radix (B) on parametrial adipose tissue weight in mice fed a high-fat diet for 8 weeks. H: high-fat diet; H+2: high-fat diet plus 2% aqueous extract of Platycodi radix; H+5: high-fat diet plus 5%aqueous extract of platycodi radix; H+0.5: high-fat diet plus 0.5% inulin; H+1: high-fat diet plus 1% inulin. Results are expressed as means ± s.e.m., n=14. *$p<0.05$, significantly different from high-fat diet group.

The accumulation of hepatic triacylglycerol caused by the high-fat diet was significantly decreased by feeding the high-fat diet containing 5% aqueous extract of Platycodi radix compared with feeding the high-fat diet alone. However, consumption of the high-fat diet plus the aqueous extract of Platycodi radix did not affect the hepatic total cholesterol concentration. To clarify the active substances in Platycodi radix, we examined the effects of the major components of Platycodi radix, the inulin and saponin fractions, on pancreatic lipase activity. Inulin had no effect on the pancreatic lipase activity, and high-fat diet-induced obesity in mice was not prevented by the administration of inulin.

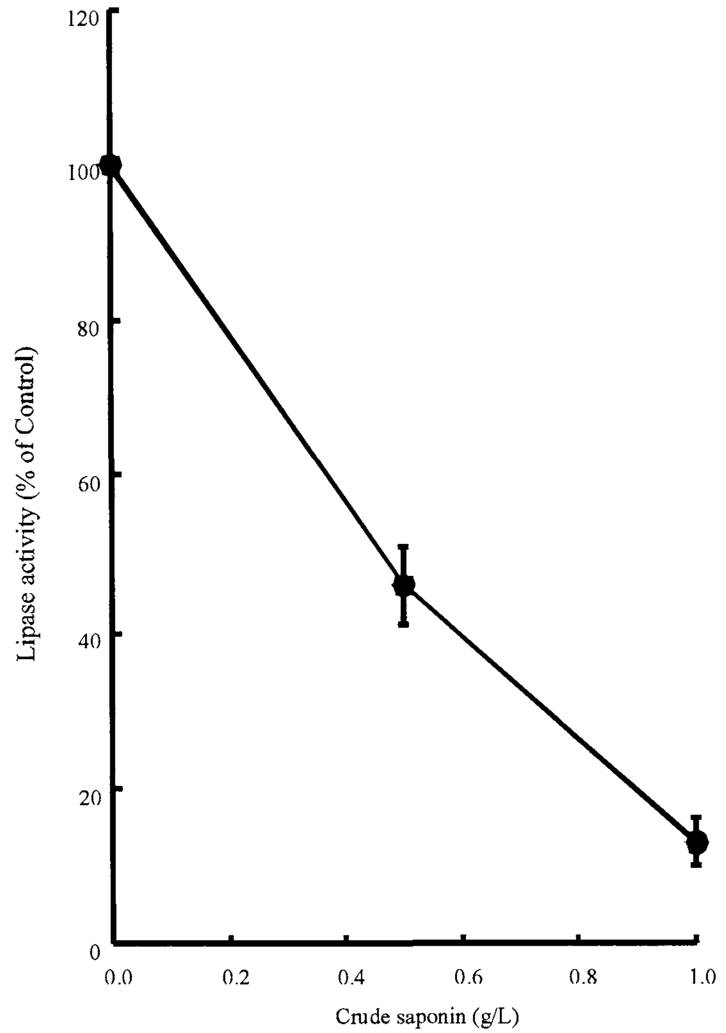



**Fig. (10)** Effect of crude saponin fraction isolated from the aqueous extract of Platycodi radix on pancreatic lipase activity *in vitro* Results are expressed as mean ± s.e.m. of four experiments.

It has been reported that various saponins isolated from foodstuffs have anti-obesity or hypolipidaemic actions [12-16]. We found that the crude saponin fractions isolated from Platycodi radix strongly inhibited the pancreatic lipase activity, Fig. **(10)**.

Therefore, it seems likely that the anti-obesity, anti-hypolipidaemic, anti-fatty liver actions of the aqueous extract of Platycodi radix may be attributed in part to its crude saponin fractions.

**Chitin-chitosan**

Chitin-chitosan is a mixture of chitin (20%) and chitosan (80%). Chitin and chitosan are polymers containing more than 5,000 acetylglucosamine and glucosamine units, respectively, and their molecular weights are over one million Daltons. Although chitin is widely distributed in natural products such as the protective cuticles of crustaceans and insects, and the cell walls of some fungi and micro-organisms, it is usually prepared from shells of crabs and shrimp. Alkaline hydrolysis (45% NaOH, 100°C) of chitin converts it to chitosan. The alkaline hydrolysate, however, contains chitosan (80%) and non-reactive chitin (20%) and is usually called 'chitin-chitosan'. Inclusion of chitin-chitosan in the diet, reduced total plasma cholesterol and inhibited the absorption of cholesterol and triacylglycerol from lymph in animal models [17-20]. Previously, we reported that chitin-chitosan reduced blood pressure elevated by NaCl intake, and augmented the cytolytic activity of mouse lymphocytes [21-22]. In the course of those experiments, we found that chitin-chitosan might inhibit the intestinal absorption of dietary fat by inhibiting hydrolysis of the fat by pancreatic lipase. To test this possibility, we studied the effect of chitin-chitosan on pancreatic lipase activity *in vitro* and measured the fat balance by determination of fat excretion in the feces of the mice fed a high-fat diet or high-fat diet plus chitin-chitosan for three days. Moreover, the present investigation was designed to clarify whether or not chitin-chitosan prevented obesity induced by feeding a high-fat diet for a longer term (9 weeks).

As shown in Fig. (**11a**), chitin-chitosan inhibited the pancreatic lipase activity dose-dependently between the concentrations of 6.25 μg/ml and 200 μg/ml in the assay system, using triolein emulsified with lecithin. For characterization of the mechanism involved in the inhibition of pancreatic lipase by chitin-chitosan, the enzyme activity was assayed at various concentrations of lecithin-emulsified triolein and in the presence of increasing concentrations of chitin-chitosan. A Lineweaver-Burk plot of the data in Fig. (**11 b**) shows that chitin-chitosan was a competitive inhibitor. The $K_m$ and $V_{max}$ values of the lipase activity for lecithin-emulsified triolein were 6.06 μg/ml and 8.7 nmol/ml/min, respectively. The $K_i$ value of chitin-chitosan on the lipase activity in lecithin-emulsified triolein was 17.6 μg/ml. When triolein was emulsified with

gum arabic instead of lecithin, chitin-chitosan did not inhibit its hydrolysis. In addition, we examined the effects of chitin-chitosan on the lipase activity using neutral polymer Triton X-100 as emulsifier. When triolein was emulsified with Triton X-100 (final concentration: 0.25 mg/ml) instead of gum arabic, chitin-chitosan did not inhibit the hydrolysis, Fig. (**11 a**).

**Table 4.** Effect of chitin-chitosan on fat excretion into feces of mice fed a high-fat (HF) diet for three days.

| Day | Mean | | | | |
|---|---|---|---|---|---|
| | **Control group** | **HF-treated group** | **HF plus 3% chitin-chtitosan-treated group** | **HF plus 7% chitin-chtitosan-treatedgroup** | **HF plus 15% chitin-chtitosan-treated group** |
| Day 1<br>Feces (g) | 1.40 | 0.27 | 0.43 | 0.56 | 0.44 |
| ExcretionTG (μ mol/g) | 4.86 | 15.7 | 15.2 | 28.6 | 17.1 |
| Day 2<br>Feces (g) | 3.02 | 0.25 | 0.54 | 2.15 | 1.16 |
| ExcretionTG (μ mol/g) | 2.56 | 19.9 | 40.4 | 46.2 | 49.1 |
| Day 3<br>Feces (g) | 1.64 | 0.18 | 0.35 | 1.18 | 1.04 |
| ExcretionTG (μ mol/g) | 3.26 | 41.8 | 50.2 | 55.3 | 59.3 |

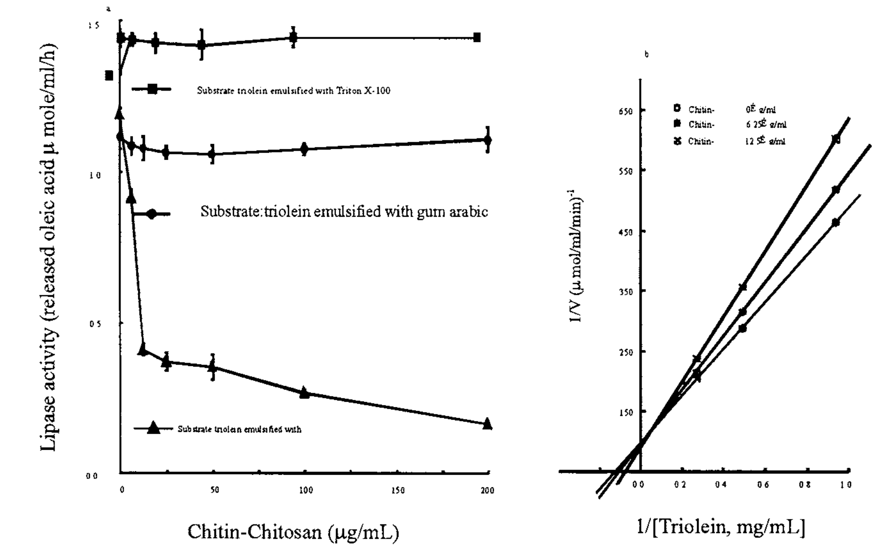
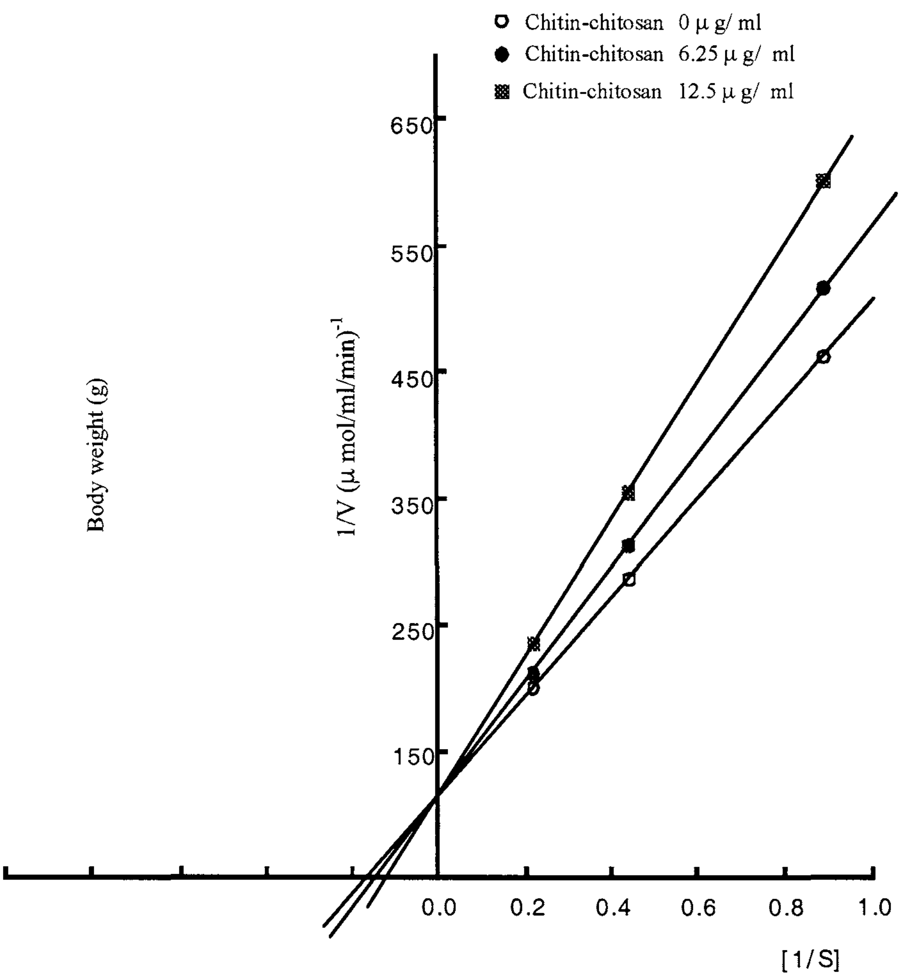



**Fig. (11)** Effects of chitin-chitosan on pancreatic lipase activity. a: Results are expressed as means ± s.e.m. of four experiments. b: Lineweaver-Burk plots of the released oleic acid with lecithin-emulsified triolein as substrate in the presence of various concentrations of chitin-chitosan.

These results suggest that the inhibitory effects of chitin-chitosan on pancreatic lipase activity may be mediated by the interaction between substrate and enzyme through lecithin. Moreover, as shown in Table 4,

the feeding of a high-fat diet plus 7% or 15% chitin-chitosan enhanced fat excretion in feces and inhibited absorption of the ingested fat in the balance study. Fig. (12) shows the changes in body weights of the

b




groups during the experiments. The final parametrial adipose tissue and liver weights of the groups are shown in Fig. (13) and Table 5, respectively. Feeding a high-fat diet containing 40% beef tallow for nine weeks caused significant increases in body weight at 4-9 weeks and in final liver and parametrial adipose tissue weights, compared to the normal diet group (laboratory pellet chow), (Fig. (12), Fig. (13) and Table 5).

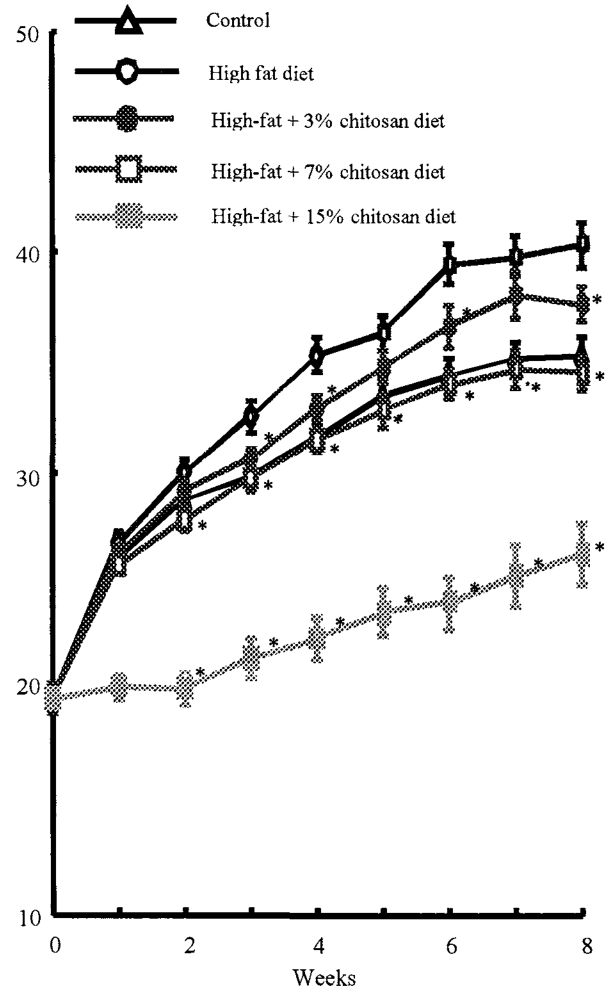



**Fig. (12)** Effect of chitin-chitosan on body weight in mice fed a high-fat diet for 9 weeks. Values are means ± s.e.m. of 13 mice.*$p<0.05$, significantly different from high-fat diet-treated group.

Feeding a high-fat diet containing 3%, 7% or 15% chitin-chitosan significantly reduced the increase in body weight at 4-9 weeks, Fig. (**12**), and the final parametrial adipose tissue weight compared to feeding a high-fat diet, Fig. (**13**). In the experiments, it was found that feeding a high-fat diet caused hyperlipidaemia, with elevations of serum

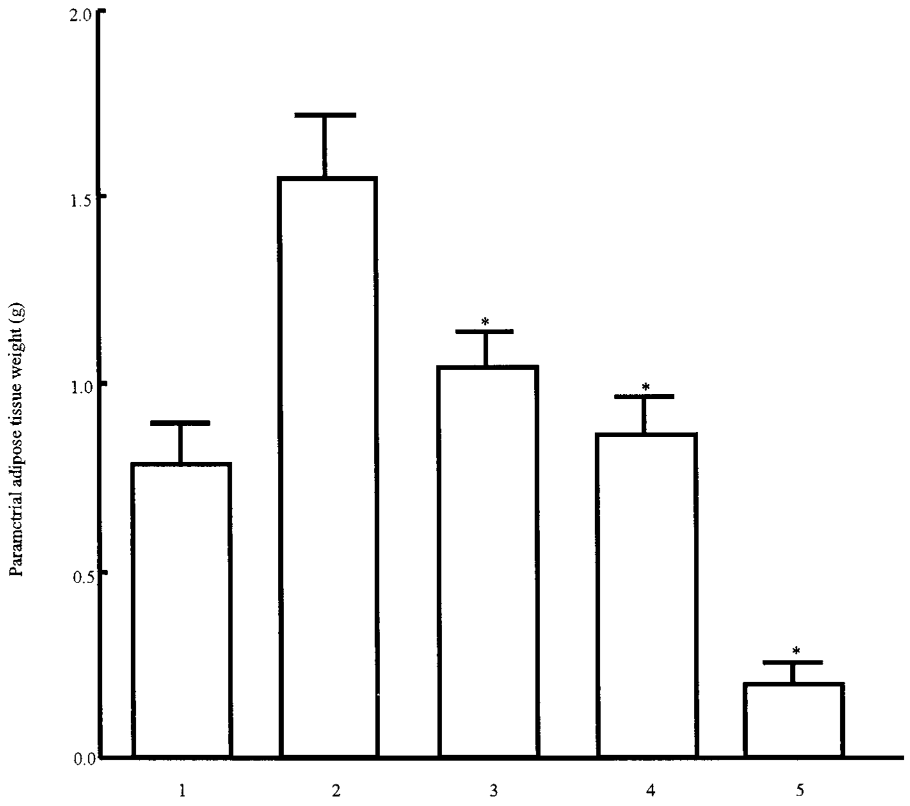



**Fig. (13)** Effects of chitin-chitosan on parametrial adipose tissue weight in mice fed a high-fat diet for 9 weeks. 1=Control group; 2=High-fat diet-treated group; 3=High-fat diet plus 3% chitin-chitosan treated groups; 4=High-fat diet plus 7% chitin-chitosan-treated group; 5=High-fat diet plus 15% chitin-chitosan-treated group. Values are means ± s.e.m. of 13 mice. *p<0.05, significantly different from high-fat diet-treated group.

triacylglycerol and total cholesterol, and caused fatty liver with accumulation of triacylglycerol and total cholesterol in the liver. Serum triacylglycerol was significantly reduced by feeding a high-fat diet containing 3%, 7% or 15% chitin-chitosan compared to feeding a high-fat diet (Table 6). Serum total cholesterol was also reduced by feeding the 7% and 15% chitin-chitosan diets (Table 6). Feeding the 3% and 15% chitin-chitosan diets reduced the serum free fatty acid level compared to feeding the high-fat diet (Table 6). The oral administration

**Table 5.** Effect of chitin-chitosan on liver weight, hepatic triacylglycerol and total cholesterol in mice fed a high-fat (HF) diet.

| | Mean | | | | |
|---|---|---|---|---|---|
| | Control group | HF-treated group | HF plus 3% chitin-chitosan-treated group | HF plus 7% chitin-chitosan-treated group | HF plus 15% chitin-chitosan-treated group |
| Liver weight ( g/100g body weight) | 4.01 | 5.864 | 5.21 | 5.21 | 4.93 |
| Triacylglycerol (μ mol/g) | 11.47 | 108.73 | 72.54 | 59.44 | 52.61 |
| Total cholesterol (μ mol/g) | 6.33 | 12.37 | 9.74 | 8.68 | 8.53 |

of 3%, 7% or 15% chitin-chitosan significantly reduced the liver weight, and prevented the accumulation of liver triacylglycerol and total cholesterol caused by a high-fat diet (Table 5). In addition, in the long-term (9 weeks) experiment, chitin-chitosan significantly reduced both

**Table 6.** Effect of chitin-chitosan on serum triacylglycerol, total cholesterol and free fatty acid in mice fed a high-fat (HF) diet.

| | Mean | | | | |
|---|---|---|---|---|---|
| | Control group | HF-treated group | HF plus 3% chitin-chitosan-treated group | HF plus 7% chitin-chitosan-treated group | HF plus 15% chitin-chitosan-treated group |
| Triacylglycerols(mM) | 1.07 | 1.65 | 0.88 | 0.87 | 0.93 |
| Total cholesterol(mM) | 1.79 | 3.02 | 2.69 | 2.32 | 2.23 |
| Free fatty acid(mEq/l) | 0.75 | 0.98 | 0.88 | 0.90 | 0.67 |

body and parametrial adipose tissue weights at doses of 3%, 7% and 15%, whereas no differences in the energy consumed were found among the experimental groups (except the lab chow group). The mean food consumption per week per mouse during the whole experimental period was significantly ($p<0.05$) different between the laboratory chow and high-fat diet groups, being 532.3±53.2 kJ in the laboratory chow and 862.5±68.2 kJ in the high-fat diet group, but not significantly different between the high-fat and high-fat plus 3%, 7% or 15% chitin-chitosan diet groups, being 862.5±68.2 kJ (high-fat diet), 927.4±29.5 kJ (3% chitin-chitosan diet), 850.1±43.4 kJ (7% chitin-chitosan diet) and 892.4±44.3 kJ (15% chitin-chitosan diet). This indicates that chitin-chitosan prevents the high-fat diet-induced increase in body weight by affecting food absorption.

It was concluded that the anti-obesity effects of chitin-chitosan in high-

fat diet-treated mice might be partly due to the inhibition of intestinal absorption of dietary fat. Consequently, chitin-chtosan might cause an

improvement in the fatty liver and hyperlipidaemia in mice fed a high-fat diet through inhibiting the intestinal absorption of dietary fat.

**Chondroitin sulfate**

Chondroitin sulfate consists of repeating D-glucuronic acid and D-*N*-acetylgalactosamine units, and the *N*-acetylgalactosamine is substituted with a sulfate at either its 4'or 6' position, with approximately one sulfate being present per disaccharide unit. Chondroitin sulfate is present in the cartilage, bone and cornea of animals. Previously, we reported that chondroitin sulfate prepared from the nasal cartilage of salmon inhibited glucose uptake into the brush border membrane vesicles prepared from rat jejunum [23]. Based on the inhibitory action of chondroitin sulfate on glucose uptake in the small intestine, we studied the effects of chondroitin sulfate on pancreatic lipase activity and fatty acid uptake into the brush border membrane vesicles *in vitro*. In addition, we examined the effects of chondroitin sulfate on the plasma triacylglycerol levels after oral administration of lipid emulsion. Moreover, the present investigation was designed to clarify whether or not chondroitin sulfate prevented obesity induced by longer-term feeding of a high-fat diet (8 weeks).

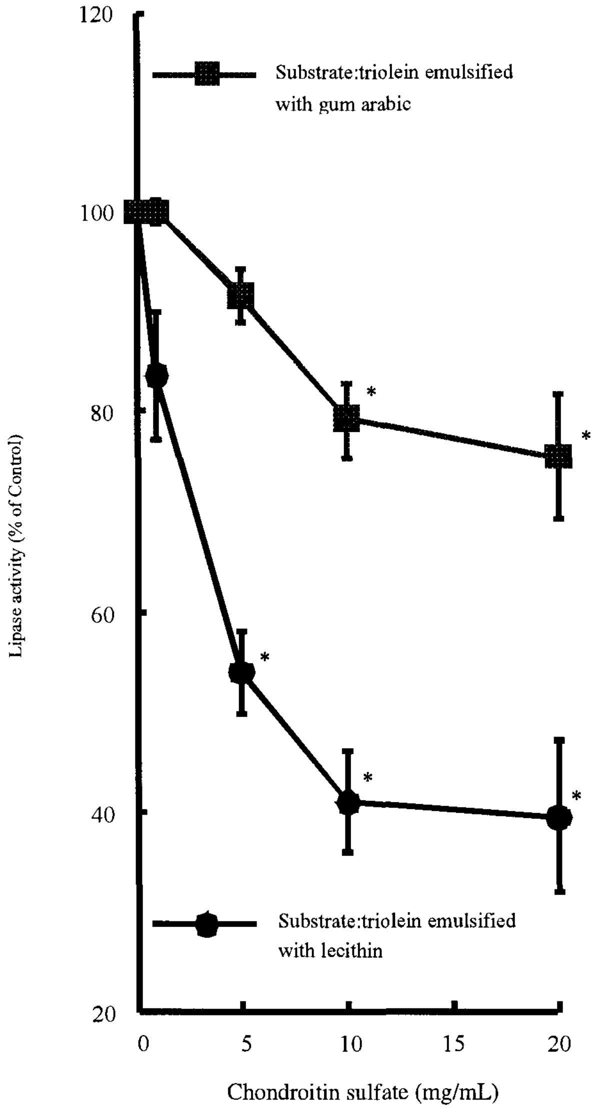



**Fig. (14)** Effects of chondroitin sulfate on pancreatic lipase activity. Results are expressed as means ± s.e.m. of four experiments. *$p<0.05$, significantly different from control.

As shown in Fig. (**14**), chondroitin sulfate inhibited the pancreatic lipase activity dose-dependently at the concentrations of 1-20 mg/ml in the assay system using triolein emulsified with phosphatidylcholine; at 10 mg/ml it inhibited triolein hydrolysis by about 60%. On the other

hand, when triolein was emulsified with gum arabic instead of phospatidylcholine, chondroitin sulfate only slightly inhibited its hydrolysis at concentrations of over 10 mg/ml; at 10 mg/ml it inhibited by about 20%.

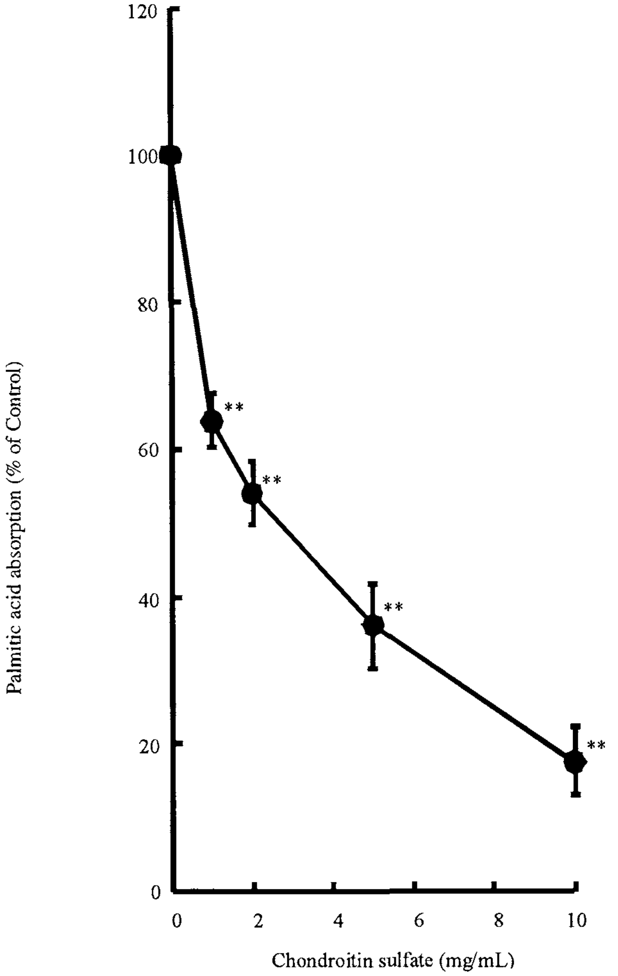



**Fig. (15)** Effects of chondroitin sulfate on palmitic acid absorption by brush border membrane vesicles. The rate of absorption is expressed as the percentage of the activity in the absence of chondroitin sulfate. Results are expressed as means ± s.e.m. of four experiments. $^{**}p<0.01$, significantly different from control.

Then, we attempted to examine the effects of chondroitin sulfate on palmitic acid and 2-monooleoylglycerol uptake into the brush border membrane vesicles of the rat small intestine *in vitro*. As shown in Fig. (15), chondroitin sulfate inhibited the incorporation of palmitic acid into

the brush border membrane vesicles dose-dependently at concentrations of 1-10 mg/ml; at 10 mg/ml it inhibited palmitic acid incorporation by about 80%. However, it did not affect the incorporation of 2-monooleoylglycerol (data not shown). Fig. (**16**) shows the time course of the plasma triacylglycerol concentrations when lipid emulsion with or without chondroitin sulfate was administered orally to rats. Two hours after chondroitin sulfate administration the plasma triacylglycerol concentration decreased significantly compared with control. No elevation of the plasma total cholesterol or free fatty acid level was caused by oral administration of the lipid emulsion (data not shown). Based on these results, we examined the effects of chondroitin sulfate on food consumption, and body, parametrial adipose tissue and liver weights in mice fed a high-fat diet for 8 weeks. Since chondroitin sulfate could not be absorbed by oral administration [24], the amount of chondroitin sulfate intake was not entered in the energy intake calculations. The mean food consumption per week per mouse at 4-8 weeks was significantly ($p<0.05$) different between the control group and high-fat diet groups, being 533.9±19.1 kJ/week/mouse in the control group and 1026.0±38.0 kJ/week/mouse in the high-fat diet group, but not significantly different among the high-fat plus chondroitin sulfate (3%, 7% or 13%) groups, being 1026.0±38.0 kJ/week/mouse (high-fat diet group), 1074.0±19.7 kJ/week/mouse (high-fat diet plus 3% chondroitin sulfate group), 990.5±16.0 kJ/week/mouse (high-fat diet plus 7% chondroitin sulfate group) and 1017.0±20.3 kJ/week/mouse (high-fat diet plus 13% chondroitin sulfate group).

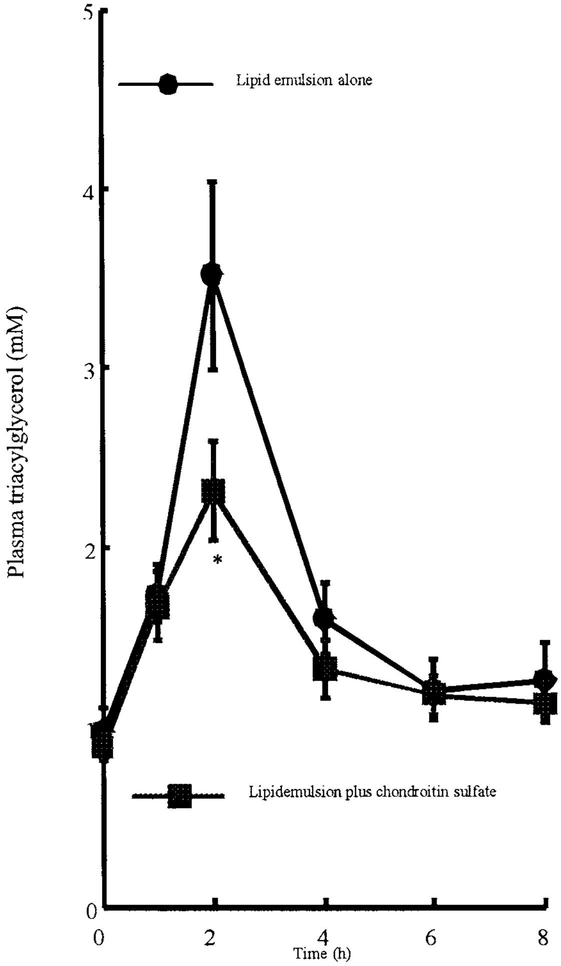



**Fig.** (16) Effects of chondroitin sulfate on rat plasma triacylglycerol levels after oral administration of lipid emulsion. Each point represents the means ± s.e.m. of 10 rats. *$p<0.05$, significantly different from lipid emulsion alone-treated group.

Fig. (**17**) and Fig. (**18**) show the changes in body weights, liver and parametrial adipose tissue weights of the groups during the experiment. Feeding a high-fat diet for 8 weeks caused significant increases in body weight at 2-8 weeks, and in the liver and parametrial adipose tissue weights, compared to the control diet group (laboratory pellet chow). The body weight at 3-8 weeks and parametrial adipose tissue and liver

weights were significantly reduced by feeding a high-fat diet containing 7% or 13% chondroitin sulfate compared to feeding a high-fat diet alone. Feeding a high-fat diet containing 3% chondroitin sulfate slightly, but not significantly reduced the body weight. However, feeding a high-fat diet containing 3% chondroitin sulfate significantly reduced the parametrial adipose tissue weight. As shown in Fig. (**19**), the diameter of fat cells was significantly greater in the high-fat diet group than in the control group, and chondroitin sulfate completely prevented the high-fat diet-induced increase in cell diameter. Moreover, as shown in Tables 7 and 8, feeding a high-fat diet caused hyperlipidaemia with elevations of plasma triacylglycerol (2.3-fold), total cholesterol (1.4-fold) and free fatty acid (1.5-fold), and caused fatty liver with accumulation of triacylglycerol (5.8-fold) and total cholesterol (1.4-fold). Plasma triacylglycerol and total cholesterol were significantly reduced by feeding a high-fat diet containing 3%, 7% or 13% chondroitin sulfate compared to feeding a high-fat diet alone: 68%, 70% or 62% for triacylglycerol and 80%, 79% or 70% for total cholesterol at 3%, 7% or 13% chondroitin sulfate diet, respectively (Table 7). Plasma free fatty acid was also significantly reduced by treatment with 13% chondroitin sulfate compared to feeding a high-fat diet alone (about 84%). Chondroitin sulfate at 3%, 7% and 13% also prevented the accumulation of hepatic triacylglycerol and total cholesterol caused by a high-fat diet:

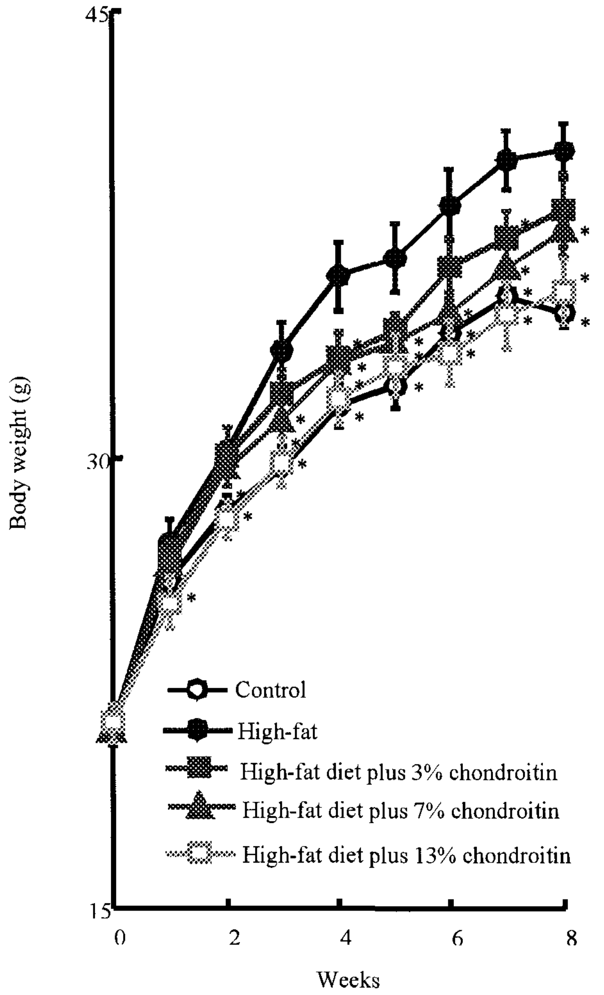



Fig. (17) Effects of chondroitin sulfate on body weight in mice fed a high-fat diet for 8 weeks. Results are expressed as means ± s.e.m. of 10-15 mice. *$p<0.05$, significantly different from high-fat diet-treated group.

76%, 58% or 55% for triacylglycerol and 75%, 71% or 65% for total cholesterol, respectively, compared to feeding a high-fat diet alone (Table 8).

Summarizing all these results, the reduction of fat storage and the antihyperlipidaemic action of chondroitin sulfate might be due to the inhibition of small intestinal absorption of dietary fat through the

inhibition of pancreatic lipase activity and fatty acid uptake through the brush border membranes.

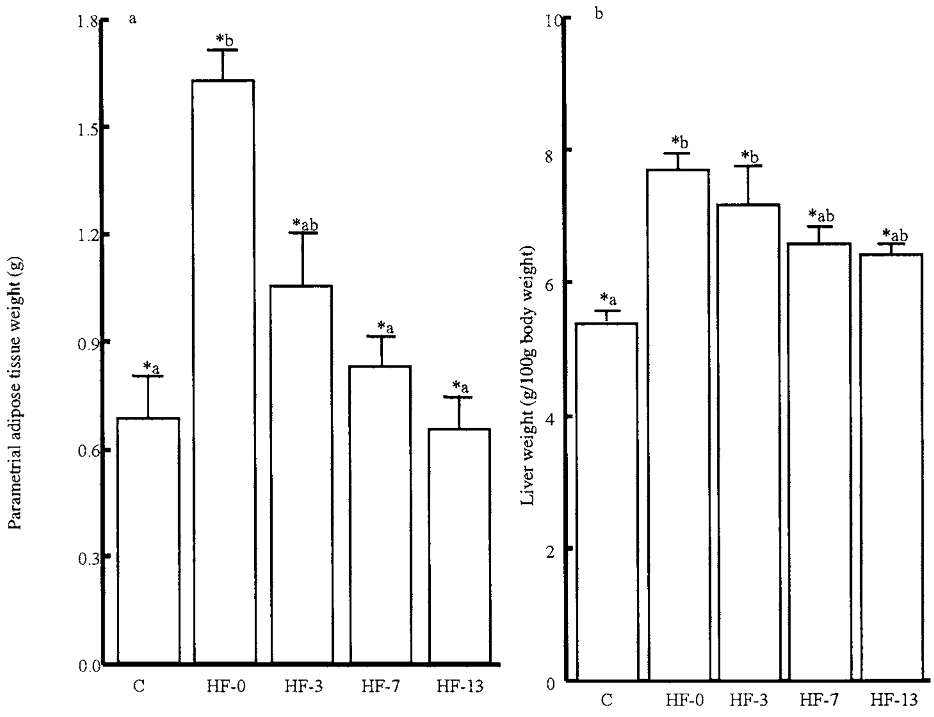



**Fig. (18)** Effects of chondroitin sulfate on parametrial adipose tissue (a) and liver (b) weights in mice fed a high-fat diet for 8 weeks. C, control; HG-0, high-fat diet; HF-3, high-fat diet plus 3% chondroitin sulfate; HF-7, high-fat plus 7% chondroitin sulfate; HF-13, high-fat diet plus 13% chondroitin sulfate; Results are expressed as means ± s.e.m. of 10-15 mice. *a:$p<0.05$, significantly different from high-fat diet-ftreated group. *b: $p<0.05$, significantly different from control group.

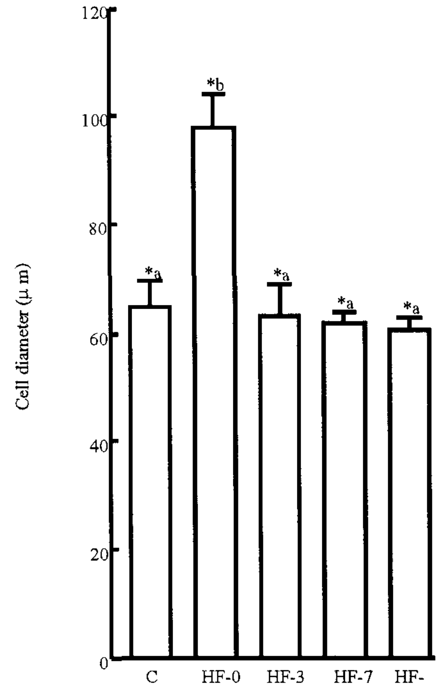



**Fig. (19)** Effects of chondroitin sulfate on the diameter of adipose cells of mice fed a high-fat diet for 8 weeks. C, control; HF-0, high-fat diet; HF-3, high-fat diet plus 3% chondroitin sulfate; HF-7, high-fat diet plus 7% chondroitin sulfate; HF-13, high-fat diet plus 13% chondroitin sulfate; Each column represents the means ± s.e.m. of four to six separate assays. *a: $p<0.05$, significantly different from high-fat diet-treated group. *b: $p<0.05$, significantly different from control group.

**Table 8.** Effects of chondroitin sulfate on hepatic triacylglycerol and total cholesterol in mice fed a high-fat diet (HF) for 8 weeks.

| | Mean | |
|---|---|---|
| | **Triacylglycerol (μ mol/g)** | **Total cholesterol (μ mol/g)** |
| Control | 21.24 | 8.35 |
| HFgroup | 124.25 | 11.74 |
| HFplus 3% chondroitin sulfate-treated | 94.18 | 8.83 |
| HFplus 7% chondroitin sulfate-treated | 72.66 | 8.28 |
| HFplus 13% chondroitin sulfate-treated | 68.65 | 7.58 |

# REFERENCES

[1] Verger, R. Pancreatic lipase; Borgstrom, B. and Brockman HL. Ed.; Lipase. Elsevier: Amsterdam, **1984**; pp. 83-150.

[2] Tsujita, T.; Matsuura, Y.; Okuda, H.; *J. Lipid Res.*, **1996**, 37, 1481-1487.

[3] Hill, JO.; Peters, JC.; Lin, D.; Yakubu, F.; Greene, H.; Swift, L.; *Int. J. Obes.*, **1993**, 17, 223-236.
[4] Kimura, Y.; Okuda, H.; Mori, K.; Okuda, T.; Arichi, S.; *J Japan Soc Nutri Food Sci*, **1984**, 37, 223-232.
[5] Okuda, T.; Kimura, Y.; Yoshida, T.; Hatano, T.; Okuda, H.; Arichi, S.; *Chem. Pharm. Bull.*, **1983**, 31, 1625-1631.
[6] Han, L-K.; Li, J.; Sumiyoshi, M.; Tsujita, T.; Kimura, Y.; Okuda, H.; *J. Trad. Med.*, **1999**, 16, 66-71.
[7] Drent, ML.; Popp, SC.; Ader, HJ.; Jansen, JB.; van der Veen, EA.; *Obes Res.*, **1995**, 3, 573-581.
[8] Dren, ML.; van der Veen, EA.; *Obes Res.*, **1995**, 3 (Suppl), 623s-625s.
[9] Dren, ML.; Larsson, I.; William, OT.; Quaade, F.; Czubayko, F.; von Bergmann, K.; Strobel, W.; van der Veen, EA.; *Int. J. Obes.*, **1995**, 19, 221-226.
[10] Hauptman, JB.; Jeunet, FS.; Hartmann, D.; *Am J clin Nutr*, **1992**, 55 (Suppl 1), 309s-313s.
[11] Kim, K.-S.; Ezaki, O.; Ikemoto, S.; Itakura, H.; *J. Nutr. Sci. Vitaminol*, **1995**, 41, 485-491.
[12] Kawano-Takahashi, Y.; Ohminami, H.; Okuda, H.; Kitagawa, I.; Yoshikawa, M.; Arichi, S.; Hayashi, T.; *Int. J. Obes.*, **1986**, 10, 293-302.
[13] Kimura, Y.; Okuda, H.; Arichi, S.; Takemoto, T.; *Shoyakugaku Zasshi*, **1983**, 37, 272-275.
[14] Oakenfull, D. G.; Fenwick, D. E.; Hood, R. L.; Topping, D. L.; Iilman, R. L.; Storer, G. B.; *Br. J. Nutr.*, **1979**, 42, 209-216.
[15] Sirtori, C. R.; Agradi, E.; Conti, F.; Mantero, O.; Gatti, E.; *Lancet*, **1977**, 1, 275-277.
[16] Yamamoto, M.; Kumagai, A.; Yamamura, Y.; *Arzneim.-Forsch.*(Drug Research), **1975**, 25, 1240-1243.
[17] Ikeda, I.; Tomari, Y.; Sugano, M.; *J. Nutr.*, **1989**, 119, 1383-1387.
[18] Sugano, M.; Watanabe, S.; Kishi, A.; Izume, M.; Ohtakara, A.; *Lipids*, **1988**, 23, 187-191.
[19] Ebihara, K.; Schneeman, BO.; *J.Nutr.*, **1989**, 119, 1100-1106.
[20] Razdan, A.; Petterson, D.; *Br. J. Nutr.*, **1994**, 72, 277-288.
[21] Zhou, A.; Matsuura, Y.; Okuda, H.; *J. Trad. Med.*, **1994**, 11, 62-64.
[22] Kato, H.; Taguchi. T.; Okuda, H.; Kondo, M.; Takara, M.; *J. Trad Med.*, **1994**, 11, 198-205.
[23] Takeda, T.; Majima, M.; Okuda, H.; *J. Jpn. Soc. Nutr. Food sci.*, **1998**, 51, 213-217.
[24] Andermann, G.; Dietz, M.; *Eur. J. Drug. Metab. Pharmacokinet*, **1982**, 7, 11-16.

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# BIOACTIVE PHENOLIC LIPIDS

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**ABSTRACT:** This review is concerned with non-isoprenoid phenolic lipids typified by compounds biosynthesised by the polyketide pathway. Botanical, biological and entomological sources of such phenolic lipids are described which contain monohydric phenols, notably cardanol and relatives, dihydric phenols such as cardols, alk(en)ylresorcinols,urushiols and phenolic acids, particularly anacardic acids. Some recently investigated mixed types of dihydric phenolic lipids are included. Separatory methods are briefly reviewed. Synthetic methods for the saturated and unsaturated members of the three main classes of interest in structure/activity studies are summarised. Biological properties of members of the three main classes are given and discussed.

## INTRODUCTION

Phenolic lipids can structurally consist of a hydrophobic alkyl chain, of isoprenoid (prenyl) or non-isoprenoid (polyketide) biosynthetic origin, attached to a hydrophilic phenolic head group. This review is concerned with the latter much larger group of non-isoprenoid compounds in which the head group is derived from a monohydric, dihydric (catechol, resorcinol or hydroquinone) or polyhydric phenol (1,2,4-trihydroxy) or from a phenolic acid related to a 6-alkyl-2-hydroxybenzoic (anacardic) or 6-alkyl-2,4-dihydroxybenzoic (orsellinic) acid. In several complex natural sources these structural types are frequently found together. The side chains comprise n-alkyl, (most usually, $C_{11}$- $C_{17}$), monoenyl, dienyl and trienyl groups (generally, $C_{15}$-$C_{17}$) with non-conjugated unsaturation commonly in the Z-configurarion, although mixed configurations abound in certain alkenylcatechols. Recent studies have also revealed compounds bearing keto and hydroxy groups in the side chain and those bearing a second hydrophobic group in the ω-position of the side chain.

This great variety of types is summarised in the section on natural occurrence. Since the appearance of previous reviews on phenolic lipids

[1,2], resorcinolic lipids [3] and anacardic acids [4,5] in the last decade, a number of new sources have been discovered. In addition, simpler isolation techniques, some new approaches to methods of synthesis have been reported which are of relevance in structure/property studies. There have been extensive studies on the biological properties of many types of phenolic lipid. All these aspects are addressed in this account.

## OCCURRENCE OF PHENOLIC LIPIDS

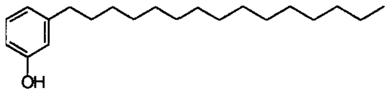
Historically, it was thought that the members of phenolic lipids are limited only to the plant kingdom and were present only in certain plant families. The first species in which the members of phenolic lipids were found was *Ginkgo biloba* (*Ginkgoaceae*) [6-10]. Later, the presence of phenolic lipids was shown also in other species, among them those belonging to the family *Anacardiaceae*, which are an important source of practically all types of phenolic lipids (alkylphenols, alkylresorcinols, anacardic acids and alkylcatechols). For example, the cashew (*Anacardium occidentale* L.) and the processing of cashew nuts is the main source of phenolic lipids for the formaldehyde-polymer industry. Aspects of *Anacardium occidentale* in relation to synthesis, semi-synthesis and chemical industry have been reviewed Tyman [1,11], as well as a recent book [2]. Monohydric alkylphenols were described in *Anacardium occidentale* (cashew) by Backer and Haack [12]who named them **anacardol** and Harvey and Caplan [13] who gave to it name of **cardanol**. The occurrence of monohydric phenolic lipids is shown in Table 1.

**Table 1. Occurrence of monohydric phenolic lipids (cardanol type)**

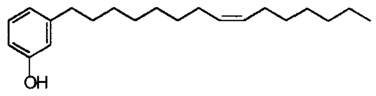
| Source | Family | Genus | References | Trivial name |
|---|---|---|---|---|
| Higher plants | *Anacardiaceae* | *Anacardium* | [12,14] | cardanol<br>anacardol |
| | | *Campnosperma* | [15] | campnospermanol |
| | | *Pentaspadon* | [16] | |
| | *Ginkgoaceae* | *Ginkgo* | [12,17,18] | ginkgol |
| | *Myrisiticaceae* | *Myristica* | [19,20] | promalabaricones |
| | *Orchidaceae* | *Habenaria* | [21] | habenariol |
| Bacteria | *Flexibacteraceae* | *Flexibacter* | [22] | flexirubin |
| Mycobacteria | *Mycobacteriaceae* | *Mycobacterium* | [23,24] | |
| Spongi | *Plakinidae* | *Plakinastrella* | [25] | elenic acid |
| | | *Placortis* | [26] | plakinidone |
| Mollusces | *Aglajidae* | *Navanax* | [27] | navenone |

Besides typical cardanol-like structure, Sleeper and Fenical have identified in 1977 navenone, an alkylphenol derivative as a component of trail pheromone of the mollusk *Navanax inermis* [27]. Another alkylphenolic derivative, plakinidone, has beed isolated in 1991 from a Caribbean sponge *Plakortis angulospiculatus* [26]. In 1995 the carboxylic acid derivative of alkylphenol - elenic acid was isolated from the Indonesian sponge Plakinastrella [25]. The structures and formulas of known monohydric phenolic lipids are presented in Fig. (1).

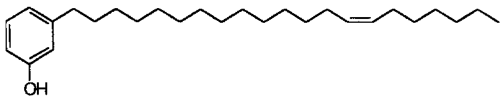
Fig. (1) Structures of known monohydric phenolic lipids



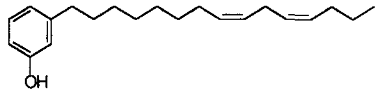

(1) [12,14]



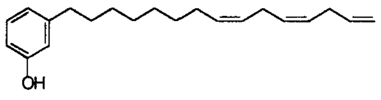

(2) [12,14,16]



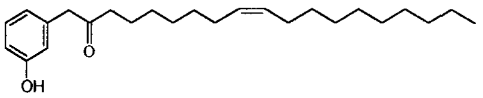

(3) [18]



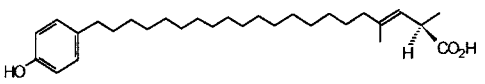

(4) [12,14,16]



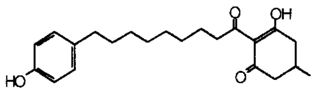

(5) [12,14]




(6) [15]

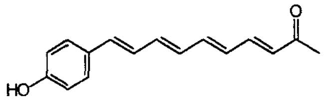



(7) [25]

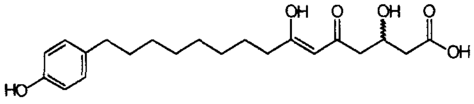



(8) [20]

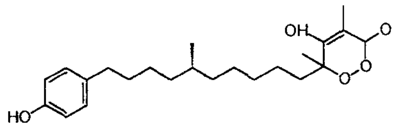
Fig. (1) cont.



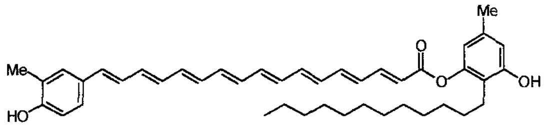

(**9**) [27]



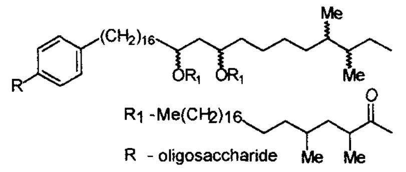

(**10**) [19,20]



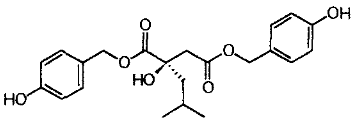

(**11**) [26]




(**12**) [22]




(**13**) [23,24]




(**14**) [21]

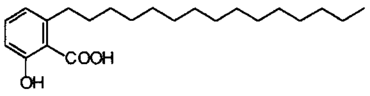
Structurarly related to this group of phenolic lipids are the compounds represented by phenolic acids and the derivatives of the anacardic acids (alkylphenolic acids). Biogenetically these acids are considered as precursors of monohydric alkylphenols that are formed from respoctive acids by decarboxylation. These compounds are distributed in many botanical families. The sources have been listed in Table 2 together with respective references. Most of the interest has been focused on anacardic acid from *Anacardium occidentale* and its unsaturated constituents. These monohydric phenolic acids are most abundant in the shell of the cashew to the extent of 20-25% [28] by contrast with other sources. Anacardic acid C15:1 isolated from *Ginkgo biloba* has acquired the name of ginkgolic acid [17].

**Table 2. Occurrence of monohydric alkylphenolic acids (anacardic acid type)**

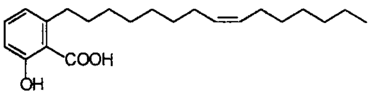
| Source | Family | Genus | References | Trivial names |
|---|---|---|---|---|
| **Higher plants** | ***Anacardiaceae*** | ***Anacardium*** | [4,29-31] | anacardic acids<br>anagigantic acid |
| | | ***Pentaspadon*** | [16] | pelandiauic acids |
| | | ***Ozoroa*** | [32] | |
| | | ***Pistacia*** | [33] | |
| | | ***Spondias*** | [34] | |
| | ***Ginkgoaceae*** | ***Ginkgo*** | [17] | ginkgolic acid |
| | ***Myrisiticaceae*** | ***Knema*** | [35] | |
| | ***Araceae*** | ***Philodendron*** | [36] | |
| | ***Compositae*** | ***Chrysanthemum*** | [37] | |
| | ***Geraniaceae*** | ***Pelargonium*** | [38-40] | frutesins |
| | ***Olacaceae*** | ***Schoepfia*** | [41] | |
| **Lichens** | | ***Sphaerophorus*** | [42] | microphyllinic acid |
| **Fungi** | ***Basidiomycetes*** | ***Merulius*** | [43] | merulinic acids |

The most common members are simple 2-hydroxy-6-[alk(en)yl] benzoic acids with C13, C15 and C17 side chain. The majority have a C15 aliphatic chain. Side chains may be saturated or contains from 1 to 3 double bonds that are, without exception, in the Z configuration. Some homologues with the even carbon atom chain length (C10, C12) have been found. The C22 and C24 acids, referred to by workers with *Pelargonium xhortorum* and from unripe *Ginkgo biloba* fruits [18,38,40,44] have in reality C15 and C17 side chains, since the six C atoms of the ring and one carboxyl group have been included into the atomic carbon assignment. The structures of anacardic acids and their derivatives are shown in Fig (2).

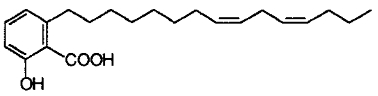
Fig. (2). Structures of known monohydric alkylphenolic acids (anacardic acid-type)



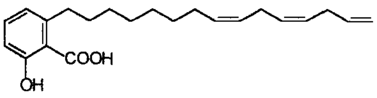

(1) [4,29,30]




(2) [4,29,30]

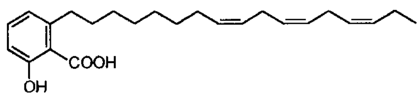



(3) [4,29,30]

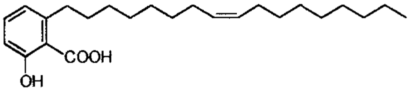



(4) [4,29-31]

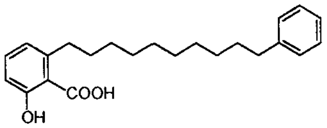
Fig. (2). Cont.



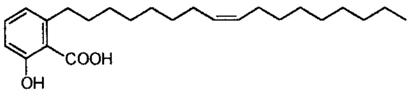

(5) [45]



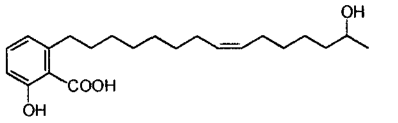

(6) [16]



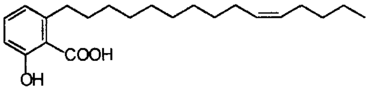

(7) [35]



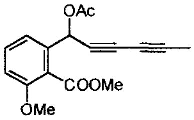

(8) [43]



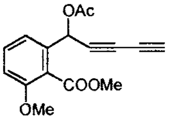

(9) [43]



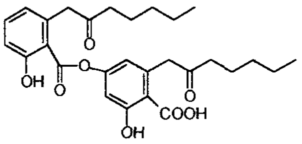

(10) [41]




(11) [37]




(12) [37]




(13) [46]

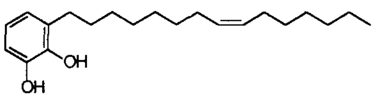
Dihydric phenolic lipids are the most abundant phenolic lipids. They are of the cardol-or urushiol-type, i.e. with hydroxyl groups in the *metha* position (1,3-dihydroxy) or in the *ortho* (1,2-dihydroxy) position respectively. Urushiol-type, dihydroxy phenolic lipids, are practically restricted to the *Anacardiaceae* family (Rhus genus) of poisonous plants. The most well-known are poison ivy, poison oak and the Indian marking nut. The sap from various plants belonging to *Anacardiaceae* from which Japanese, Chinese, Burmese, Formosan and Vietnamese lacs is one of the richest source of these phenols. The occurrence of dihydric phenolic lipid of the urushiol type is depicted in Table 3.

**Table 3. Occurrence of dihydric phenolic lipids of the urushiol type**

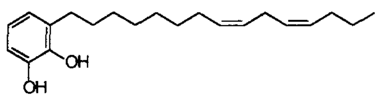
| Source | Family | Genus | References | Trivial name |
|---|---|---|---|---|
| **Higher plants** | ***Anacardiaceae*** | ***Rhus*** | [14,47-84] | urushiol, bhilavanol |
| | | ***Semecarpus*** | [82,85] | renghol |
| | | ***Melanorrhoea*** | [57,69,70,86-88] | thitsiol, laccol, urushiol |
| | | ***Gluta*** | [89] | glutarenghol |
| | | ***Smoddingium*** | [90] | |
| | ***Zingiberaceae*** | ***Zingiber*** | [91] | gingerol, shogaol, zingerone |
| | ***Myrisiticaceae*** | ***Myristica*** | [19,20] | promalabaricones malabaricones, giganteones |
| **Fungi** | ***Basidiomycetes*** | ***Gerronema*** | [92] | gerronemins |

The structures and formulas of known dihydric phenolic lipids of the urushiol type are presented in Fig. (3).

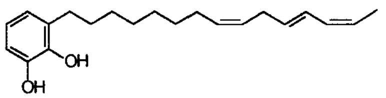
Fig. (3). Structures of known dihydric phenolic lipids of urushiol (1,2-dihydroxy) type



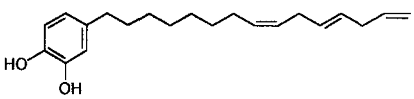

(1) [81,82,84]



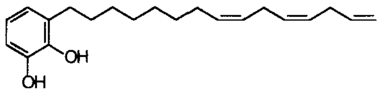

(2) [81,82]



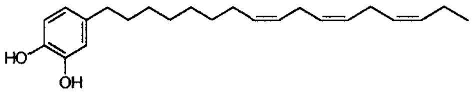

(3) [14,78]



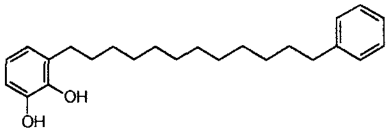

(4) [69,70,81,82,86-88]



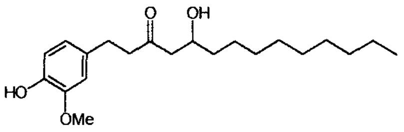

(5) [14,57]




(6) [69,70,81,82,86-88]

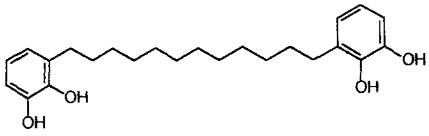



(7) [69,70,81,82,86-88]

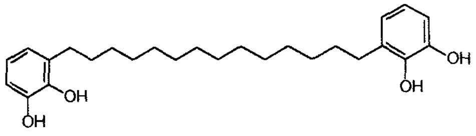



(8) [91]

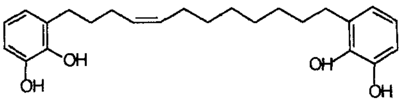
Fig. (3) Cont.



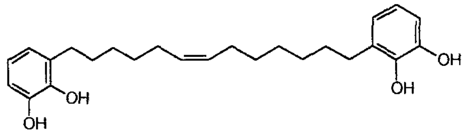
(**9**) [92]



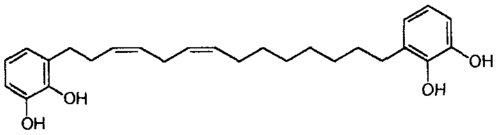
(**10**) [92]



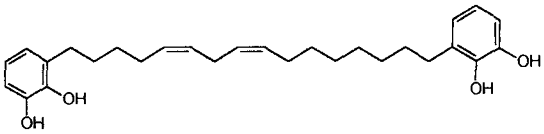
(**11**) [92]



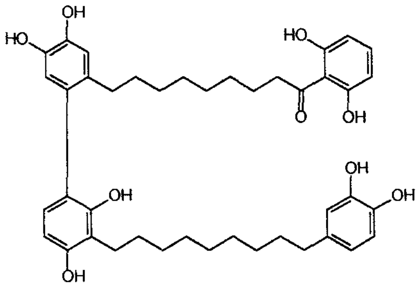
(**12**) [92]



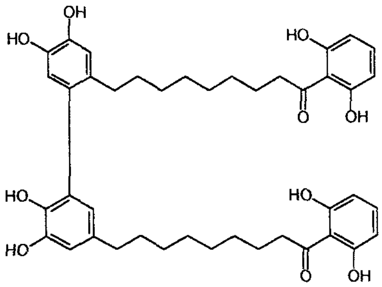
(**13**) [92]



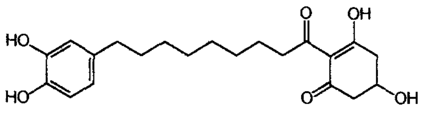
(**14**) [92]



(**15**) [19,20]



(**16**) [19,20]



(**17**) [19,20]

Dihydric phenolic lipids of the cardol type are the most abundant in plant, fungal and bacterial kingdoms. The first species in which the members of the title subclass of phenolic lipids, resorcinolic lipids, were found was *Ginkgo biloba* (*Ginkgoaceae*) [6,9,10,17]. Later, the presence of resorcinolic lipids (5-n-alk(en)ylresorcinols) was shown also in other species, first, in the *Anacardiaceae* [14], which is an important source of various phenolic lipids, not only of alkylresorcinols but also of alkylphenols and alkylcatechols. For example, the cashew and the processing of cashew nuts are the main source of phenolic lipids for the formaldehyde-polymer industry. Aspects of *Anacardium occidentale* in relation to synthesis, semi-synthesis and chemical industry have been reviewed Tyman [1,11,14] as well as a recent book [2].

Another plant family in which the occurrence of resorcinolic lipids was demonstrated is the *Gramineae* family. The pioneering work of Wenkert has demonstrated the existence of substantial amounts of 5-n-alkylresorcinols in wheat bran [93]. Later, the occurrence of resorcinolic lipid in rye [94] and barley [95] has been shown, compared with other families [96-98] and also shown among lower plants. A number of further studies have been concerned with the determination, localization and characterization of various resorcinolic lipids present in cereal grains [99-103]. Later, the occurrence of resorcinolic lipids has been demonstrated in an increasing number of plant sources.

Higher plant resorcinolic lipids include very simple homologues of the orcinol-type (1,3-dihydroxy-5-methylbenzene) phenols and a variety of homologues that are derivatives with the ring, chain or both ring and chain modified. Resorcinolic lipid molecules have a dual, aromatic and acyclic character. In most cases the side chain in resorcinolic lipids is odd numbered, which is relevant with regard to their possible biosynthetic pathway.

Since 1969 the existence of resorcinolic lipids has been demonstrated in microbial organisms [24]. Leprosols, are resorcinolic lipids derivatives which have been identified in *Mycobacterium leprae*. Later the occurrence of simple 5-alkylresorcinols has been demonstrated in strains of soil bacteria from *Azotobacter* [104-107] and *Pseudomonas* families [104-111]. Bacterial resorcinolic lipids have been thought to be related exclusively to the transformation of vegetative cells into their dormant forms, cysts [106]. However, our previous studies on other *Azotobacter* and *Pseudomonas* strains [107], as well as recent, unpublished data indicate the occurrence of resorcinolic lipids also in vegetative cells, non-

stimulated with β-hydroxybutyrate. The striking feature of microbial sources, contrary to plant materials, is the exclusive occurrence of 5-alkylresorcinol homologues with saturated chains. The occurrence of these compounds in soil bacteria, some of them related to nitrogen fixation and symbiosis with higher plants is suggestive of the evolutionary background of these compounds and a possible relationship between microbial and higher plant sources.

The natural sources in which resorcinolic lipids (simple alkylresorcinols and various types of derivatives) occur includes 11 families of the higher plants, 6 lower plant families (algae, mosses and fungi) and several bacterial families (3). A striking observation is the very seldom occurrence of resorcinolic lipids in animal organisms. In 1991 Barrow has reported the presence of alkyl and alkenyl resorcinols in marine sponge *Haliclona* sp. [112]. Since this report the occurrence of nonenyl alkylresorcinol in protozoan organism (*Climacostomum virens*) belonging to heterotrich cilliates has been reported [113].

The amount of resorcinolic lipids in plant and microbial sources varies considerably depending on the source. The most prominent source of resorcinolic lipids (cardol and methylcardol), as well as of other phenolic lipids, is the oil obtained from extraction of the shell of cashew nuts, *Anacardium occidentale,* (up to 20% of resorcinolic lipids). The oily extract from the roasting of cashew nuts (Cashew Nut Shell Liquid) is one of the most important sources of these compounds for formaldehyde polymerisation in industry [1,2,11,114]. Other plant sources contain amounts of resorcinolic lipids that vary from 0.01% to 0.1% with rye grains as the richest [101,115-120] while bacterial sources, depending on the family and strain contain up to 6% of various resorcinolic lipid derivatives [105].

In most cases resorcinolic lipids occur as mixtures of at least several homologues, generally having one to three different chain lengths and/or degrees of unsaturation. In some cases, they occur as only a few derivatives with respect to the side-chain length which can be saturated or unsaturated [121]. In certain higher plants, namely *Graminaceae*, especially cereal grains, the presence of numerous homologues ranging from $C_{13}$, to $C_{27}$, with each of these homologs in saturated, monounsaturated and diunsaturated form has been shown [101,122-127]. A similar wide spectrum of homologues exists in bacterial cells of *Azotobacter* and *Pseudomonas*, although the presence of only saturated

homologues was noted [104,106,107,110,128,129]. Apart from simple 5-n-alk(en)ylresorcinols in plant material and bacterial cells, the occurrence of various derivatives (ring or chain modified) has also been demonstrated. The chain in many cases is saturated or with one to four double bonds in a *cis* configuration. The localization of double bonds is different and depends on the side-chain length. The most frequent position of double bonds is at the $C_8$, $C_{11}$, $C_{14}$ carbon atoms (in case of $C_{15}$ homologues) making a striking comparison with the double bond location in $C_{18}$ fatty acids excluding the carboxyl group. In homologues with longer side-chains, double bonds have been localized at other carbon atoms notably at the C10 [130] although one methylene group separates each double bond as observed in unsaturated fatty acids.

The structures of over 120 identified natural resorcinolic lipid homologues are presented in Table 4 and Fig. (4) which lists the formulae of resorcinolic lipids and relevant references to previous and current studies.

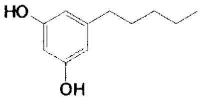
**Table 4. Occurrence of dihydric phenolic lipids of the cardol type**

| Source | Family | Genus | References | Trivial names |
|---|---|---|---|---|
| **Higher plants** | ***Anacardiaceae*** | ***Anacardium*** | [1,131,132] | cardols |
| | | ***Mangifera*** | [133-135] | |
| | | ***Melanorrhoea*** | [57,86,87] | |
| | ***Ginkgoaceae*** | ***Ginkgo*** | [7,12,17,136,137] | bilobols |
| | ***Proteaceae*** | ***Grevillea*** | [138-144] | grevillol, striatol, grebustols |
| | | ***Hakea*** | [138,145-147] | |
| | | ***Opistholepis*** | [138] | |
| | | ***Protea*** | [141,148] | |
| | | ***Persoonia*** | [138,149] | persoonol |
| | | ***Cardwellia*** | [138,149] | |
| | ***Myrsinaceae*** | ***Rapanea*** | [150] | |
| | | ***Ardisia*** | [151-155] | ardisiphenols |
| | | ***Oncostemon*** | [156] | oncostemonols |
| | ***Primulaceae*** | ***Lysimachia*** | [157] | |
| | ***Myristicaceae*** | ***Knema*** | [35,158] | |
| | | ***Virola*** | [159] | |
| | | ***Myristica*** | [160] | |
| | ***Melastomaceae*** | ***Miconia*** | [161] | miconidin |
| | ***Iridaceae*** | ***Iris*** | [162] | irisresorcinol |
| | ***Araceae*** | ***Philodendron*** | [36,163,164] | |
| | | ***Monstera*** | [163] | |
| | ***Compositae*** | ***Conyza*** | [165] | |
| | | ***Artemisia*** | [166] | |
| | | ***Baccharis*** | [167] | |
| | | ***Senecio*** | [168] | |
| | ***Leguminoseae*** | ***Ononis*** | [169-175] | |
| | | ***Genista*** | [176] | |
| | | ***Pisum*** | [176] | |

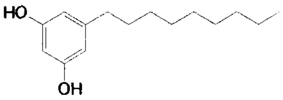
**Table 4. Cont.**

| Source | Family | Genus | References | Trivial names |
|---|---|---|---|---|
| | | ***Lathyrus*** | [176] | |
| | ***Gramineae*** | ***Triticum*** | [93,100,101,115,122,177-179] | |
| | | ***Secale*** | [94,100,101,115,122-124,177-181] | |
| | | ***Hordeum*** | [95,182] | |
| | | ***Triticale*** | [100] | |
| | | ***Sorghum*** | [183-186] | xenognosin, didydrosorgoleone |
| | | ***Oryza*** | [187-189] | |
| | | ***Agropyron*** | [190] | |
| | | ***Bromus*** | [190] | |
| | | ***Elymus*** | [190] | |
| | | ***Dactylis*** | [190] | |
| | | ***Arrhenetherum*** | [190] | |
| | | ***Alopecurus*** | [190] | |
| | | ***Festuca*** | [190] | |
| **Algae** | ***Chlorophycae*** | ***Botryococcus*** | [191] | botryococcoid ethers |
| | | ***Apatococcus*** | [192] | |
| | ***Sargassaceae*** | ***Cystophora*** | [193] | |
| | | ***Caulocystis*** | [9] | |
| **Mosses** | | ***Sphaerophorus*** | [9,46,194] | |
| | | ***Lobaria*** | [98] | |
| **Fungi** | ***Basidiomycetes*** | ***Merulius*** | [43] | merulinic acids |
| | | ***Phlebia*** | [43] | |
| | | ***Phoma*** | [195] | |
| | | ***Corticium*** | [196] | |
| | | ***Pulchericium*** | [196] | |
| | | ***Fusarium*** | [197] | |
| | | ***Verticladiella*** | [198] | |
| | | ***Albatrellus*** | [199] | albaconol |
| | | ***Streptomyces*** | [200,201] | panosialins, adipostatins |
| | ***Botryomyces*** | ***Lasiodiplodia*** | [202] | |
| | ***Hypnomycetes*** | ***Stemphylium*** | [194] | stemphol |
| **Bacteria** | ***Actinomycetales*** | ***Mycobacterium*** | [24,134] | leprosols |
| | ***Pseudomonales*** | ***Pseudomonas*** | [105,107-111] | |
| | ***Eubacteriales*** | ***Azotobacter*** | [104-107] | |
| **Spongi** | ***Haliclonidae*** | ***Haliclona*** | [112] | |
| **Protozoa** | ***Climacosto,atide*** | ***Climacostomum*** | [113] | climacostol |

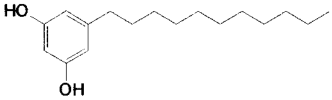
Fig. (4) Structures of known dihydric phenolic lipids of cardol (1,3-dihydroxy) type



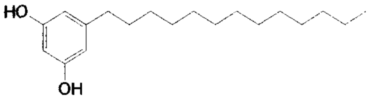
(**1**) 1,3-dihydroxy-5-pentylbenzene [98]



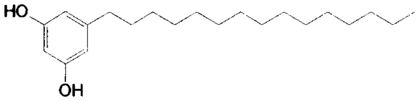
(**2**) 1,3-dihydroxy-5-nonylbenzene [138]



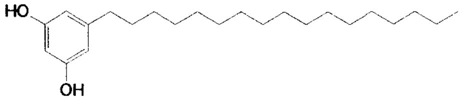
(**3**) 1,3-dihydroxy-5-undecylbenzene [148,149]



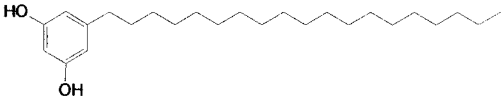
(**4**) 1,3-dihydroxy-5-tridecylbenzene [9,36,134,140-142,146,147,157,168,188]



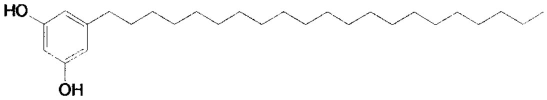
(**5**) 1,3-dihydroxy-5-pentadecylbenzene [12,85,94,100,101,122-124,134,146,165,167,168,188,189,201,203]



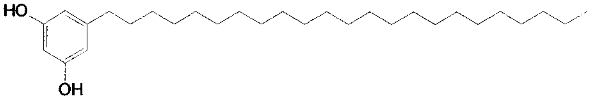
(**6**) 1,3-dihydroxy-5-heptadecylbenzene [94,101,107,122,124,125,130,134,162,165,179,187,188,190]



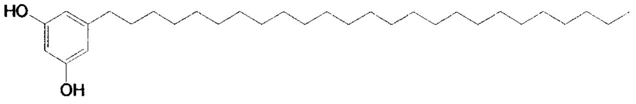
(**7**)1,3-dihydroxy-5-nonadecylbenzene [93-95,100,101,104,122-124,130,179,180,190,204]



(**8**) 1,3-dihydroxy-5-heneicosylbenzene [93-95,100,101,104,122-124,130,179,180,190,204]

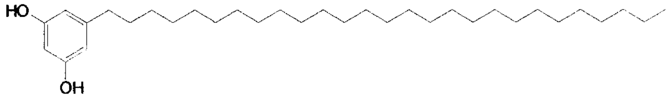


(**9**) 1,3-dihydroxy-5-tricosylbenzene [93-95,100,101,104,122-124,130,179,180,190,204]

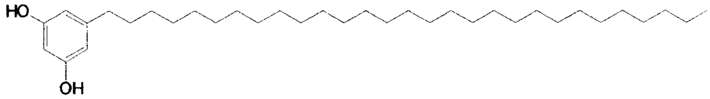


(**10**) 1,3-dihydroxy-5-pentacosylbenzene [93-95,100,101,104,122-124,130,179,190]

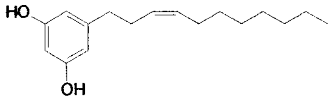
Fig. (4) cont.



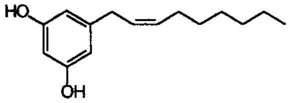
(**11**) 1,3-dihydroxy-5-heptacosylbenzene [95,181,190]



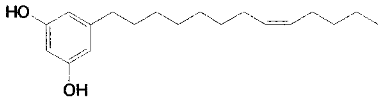
(**12**) 1,3-dihydroxy-5-nonacosylbenzene [181,190]



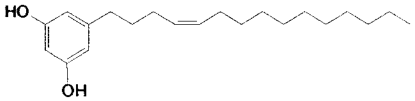
(**13**) 1,3-dihydroxy-5-(undec-3*Z*-enyl)benzene [149]



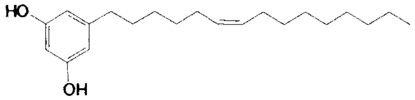
(**14**) 1,3-dihydroxy-5-(none-2Z-enyl)benzene [113]



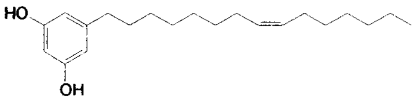
(**15**) 1,3-dihydroxy-5-(tridec-8*Z*-enyl)benzene [148,150,152,153]



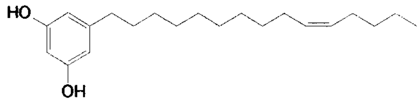
(**16**) 1,3-dihydroxy-5-(pentadec-4*Z*-enyl)benzene [112]



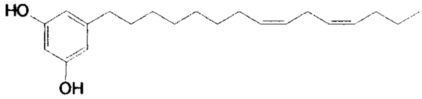
(**17**) 1,3-dihydroxy-5-(pentadec-6*Z*-enyl)benzene [112]



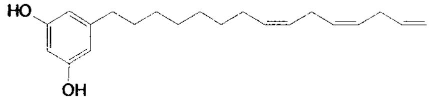
(**18**) 1,3-dihydroxy-5-(pentadec-8*Z*-enyl)benzene [1,12,17,85,132,147,150,170,171,188,203]



(**19**) 1,3-dihydroxy-5-(pentadec-10*Z*-enyl)benzene [1,12,132]

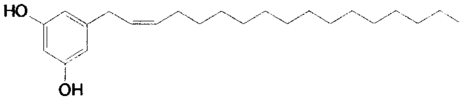


(**20**) 1,3-dihydroxy-5-(pentadec-8*Z*,11*Z*-dienyl)benzene [140,150,164]

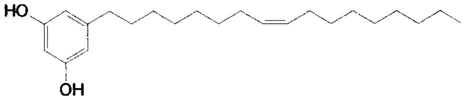


(**21**) 1,3-dihydroxy-5-(pentadec-8*Z*,11*Z*,14*Z*-trienyl)benzene [133]

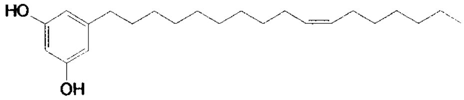
Fig. (4) cont.



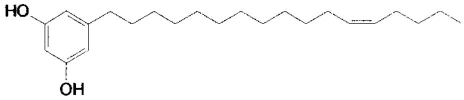
(**22**) 1,3dihydroxy-5-(heptadec-2*Z*-enyl)benzene [1,132]



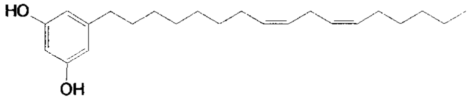
(**23**) 1,3-dihydroxy-5-(heptadec-8*Z*-enyl)benzene [145,188]



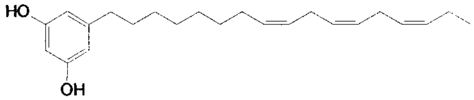
(**24**) 1,3-dihydroxy-5-(heptadec-10*Z*-enyl)benzene [162]



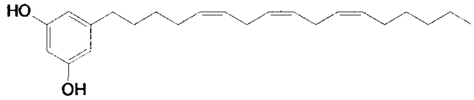
(**25**) 1,3-dihydroxy-5-(heptadec-12*Z*-enyl)benzene [130,134,187]



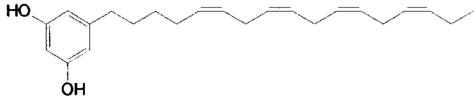
(**26**) 1,3-dihydroxy-5-(heptadec-8*Z*,11*Z*-dienyl)benzene [112,130,143,181]



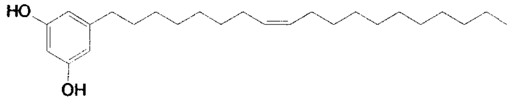
(**27**) 1,3-dihydroxy-5-(heptadec-8*Z*,11*Z*,14*Z*-trienyl)benzene [36,112]



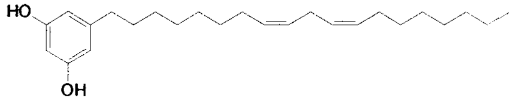
(**28**) 1,3-dihydroxy-5-(heptadec-5*Z*,8*Z*,11*Z*-trienyl)benzene [193]



(**29**) 1,3-dihydeoxy-5-(heptadec-5*Z*,8*Z*,11*Z*,14*Z*-tetraenyl)benzene [193]

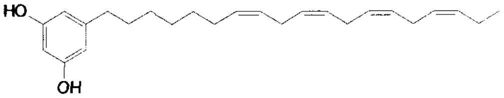


(**30**) 1,3-dihydroxy-5-(nonadec-8*Z*-enyl)benzene [181]

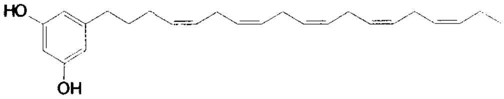


(**31**) 1,3-dihydroxy-5-(nonadec-8*Z*,11*Z*-dienyl)benzene [181]

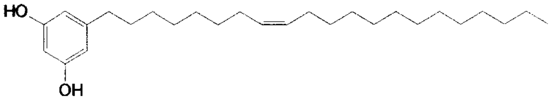
Fig. (4) cont.



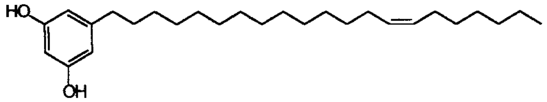
(**32**) 1,3-dihydroxy-5-(nonadec-7*Z*,10*Z*,13*Z*,16*Z*-tetraenyl)benzene [112]



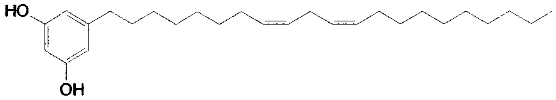
(**33**) 1,3-dihydroxy-5-(nonadec-4*Z*,7*Z*,10*Z*,13*Z*,16*Z*-pentaenyl)benzene [112]



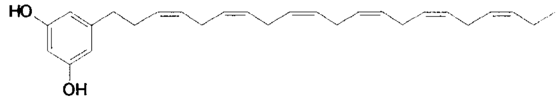
(**34**) 1,3-dihydroxy-5-(heneicos-8*Z*-enyl)benzene [181]



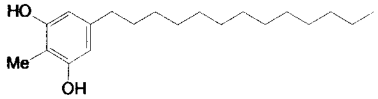
(**35**) 1,3-dihydroxy-5-(heneicos-14*Z*-enyl)benzene [18]



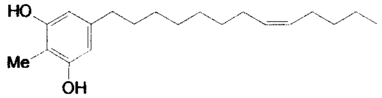
(**36**) 1,3-dihydroxy-5-(heneicos-8*Z*,11*Z*-dienyl)benzene [181]



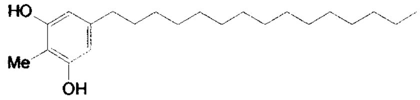
(**37**) 1,3-dihydroxy-5-(heneicos-3*Z*,6*Z*,9*Z*,12*Z*,15*Z*,18*Z*-hexaenyl)benzene [112]



(**38**) 1,3-dihydroxy-2-methyl-5-tridecylbenzene [146]

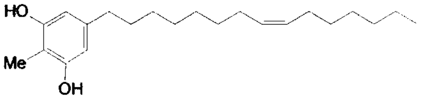


(**39**) 1,3-dihydroxy-2-methyl-5-(tridec-8*Z*-enyl)benzene [148,152,153]

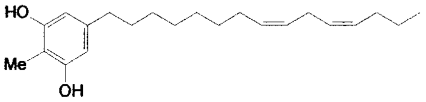


(**40**) 1,3-dihydroxy-2-methyl-5-pentadecylbenzene [1]

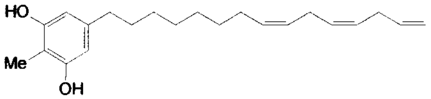
Fig. (4) cont.



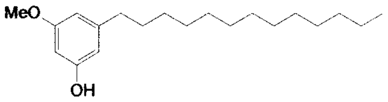

(**41**) 1,3-dihydroxy-2-methyl-5-(pentadec-8*Z*-enyl)benzene [1]



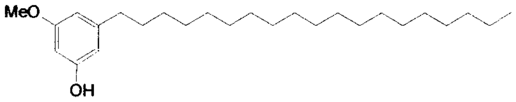

(**42**) 1,3-dihydroxy-2-methyl-5-(pentadec-8*Z*,11*Z*-dienyl)benzene [1]



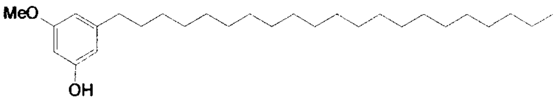

(**43**) 1,3-dihydroxy-2-methyl-5-(pentadec-8*Z*,11*Z*,14-trienyl)benzene [1]



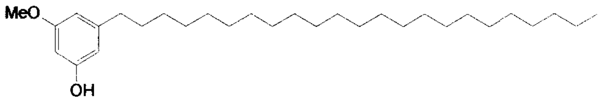

(**44**) 1-hydroxy-3-methoxy-5-tridecylbenzene [146]



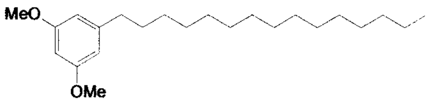

(**45**) 1-hydroxy-3-methoxy-5-nonadecylbenzene [166]



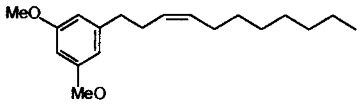

(**46**)1-hydroxy-3-methoxy-5-heneicosylbenzene [166]




(**47**) 1-hydroxy-3-methoxy-tricosylbenzene [166]

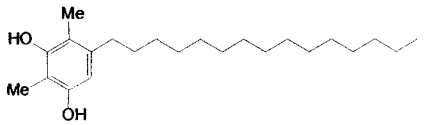



(**48**) 1,3-dimethoxy-5-pentadecylbenzene [205]

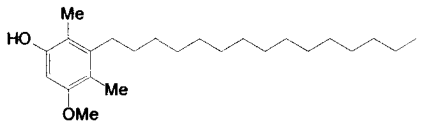



(**49**) 1,3-dimetoxy-5-(undec-3Z-enyl)benzene [138]

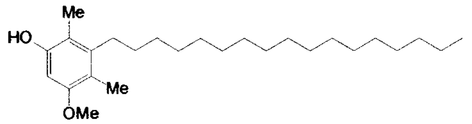
Fig. (4) cont.



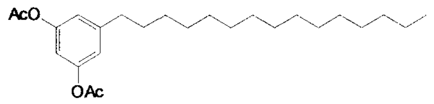

(**50**) 1,3-dihydroxy -2,6-dimethyl-5-pentadecylbenzene [24]



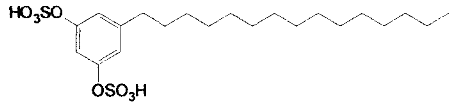

(**51**) 1-hydroxy-2,4-dimethyl-5-methoxy-3-pentadecylbenzene [24]



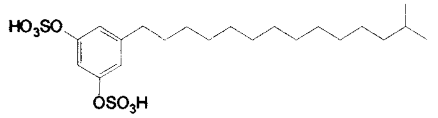

(**52**) 1-hydroxy-2,4-dimethyl-5-methoxy-3-heptadecylbenzene [24]



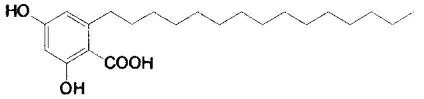

(**53**) 1,3-diacetoxy-5-pentadecylbenzene [165]



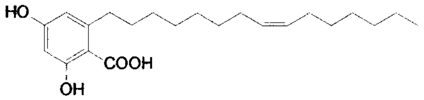

(**54**) 1.3-dihydroxy-5-pentadecylbenzene disulphate [96,200,206]




(**55**) 1,3-dihydroxy-5-isopentadecylbenzene disulphate [96,200,206]

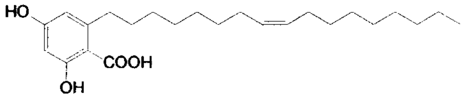



(**56**) 2,4-dihydroxy-6-pentadecylbenzoic acid [157,196,207]

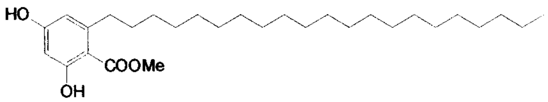



(**57**) 2,4-dihydroxy-6-(pentadec-8*Z*-enyl)benzoic acid [169-171,207]

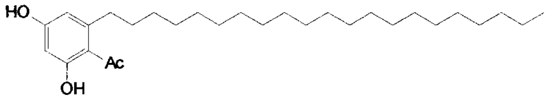
Fig. (4) cont.



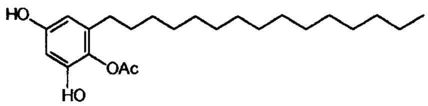

(**58**) 2,4-dihydroxy-6-(heptadec-8*Z*-enyl)benzoic acid [43]



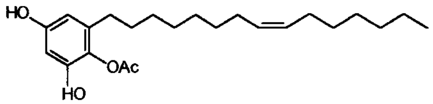

(**59**) Methyl 2,4-dihydroxy-6-heneicosylbenzoate [106,107]



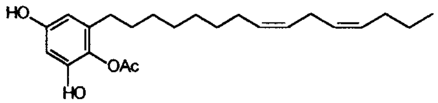

(**60**) 5-acetyl-2,4-dihydroxy-6-heneicosylbenzene [106,107]



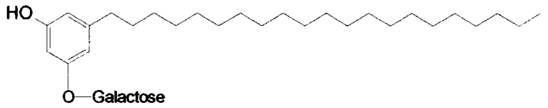

(**61**) 5-acetoxy-2,4-dihydroxy-6-pentadecylbenzene [154,155]



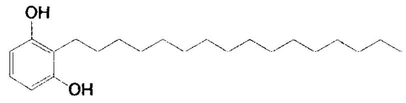

(**62**) 5-acetoxy-2,4-dihydroxy-6-(pentadec-8Z-enyl)benzene [154,155]




(**63**) 5-acetoxy-2,4-dihydroxy-6-(pentadec-8Z,11Z-dienyl)benzene [154,155]

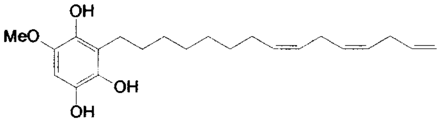



(**64**) 1-(D-galactopyranosyl)-3-hydroxy-5-heneicosylbenzene [106,107]

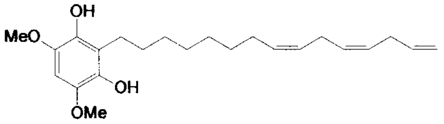



(**65**) 1,3-dihydroxy-2-hexadecylbenzene [159]

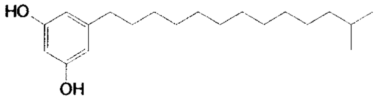
Fig. (4) cont.



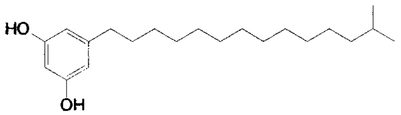
(**66**) 1 -Methoxy-3-(pentadec-8*Z*,11*Z*,14-trienyl)-2,4,5-trihydroxybenzene [183,208]



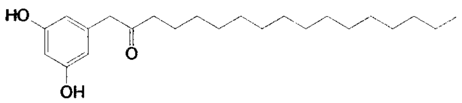
(**67**) 2,4-dihydroxy-1,5-dimethoxy-2-(pentadec-8*Z*,11*Z*,14-trienyl)benzene [184]



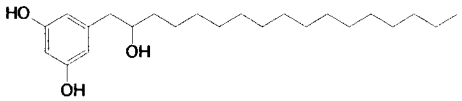
(**68**) 1,3-dihydroxy-5-isotetradecylbenzene [112]



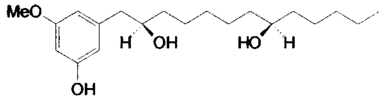
(**69**) 1,3-dihydroxy-5-isopentadecylbenzene [201]



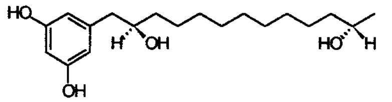
(**70**) 1,3-dihydroxy-5-(2-oxoheptadecyl)benzene [178,181]



(**71**) (2'*R*/*S*)-1,3-dihydroxy-5-(2'-hydroxyheptadecyl)benzene [107,181]

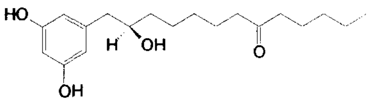


(**72**) (2'*R*), (8'*S*)-1-hydroxy-3-metoxy-5-(2',8'-dihydroxytridecyl)benzene [170]

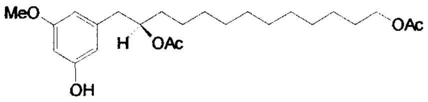


(**73**) (2'*R*), (12'*S*)-1,3-dihydroxy-5-(2',12'-dihydroxytridecyl)benzene [173]

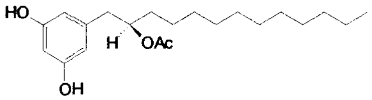
Fig. (4) cont.



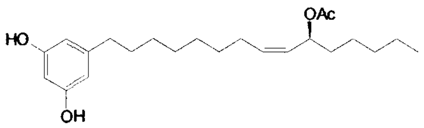
(**74**) (2'*R*)-1,3-dihydroxy-5-(2'-hydroxy-8'-oxotridecyl)benzene [170]



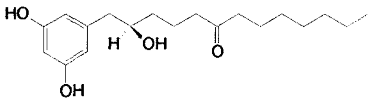
*(**75**) (2'*R*)-1,3-dihydroxy-5-(2',13'-diacetoxytridecyl)benzene [170]



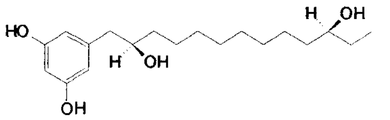
*(**76**) (2'*R*)-1,3-dihydroxy-5-(2'-acetoxytridecyl)benzene [170]



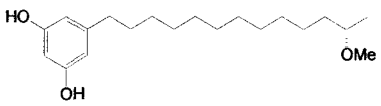
(**77**) (10'*S*)-1,3-dihydroxy-5-(10'-acetoxypentadec-8*Z*-enyl)benzene [175]



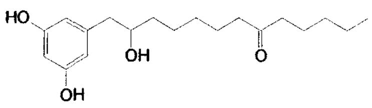
(**78**) (2'*R*)-1,3-dihydroxy-5-(2'-hydroxy-6'-oxotridecyl)benzene [174]



(**79**) (2'*R*),(11'*S*)-1,3-dihydroxy-5-(2'11'-dihydroxytridecyl)benzene [171]

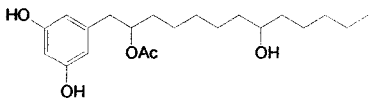


*(**80**) (12'*S*)-1,3-dihydroxy-5-(12'-methoxytridecyl)benzene [171]

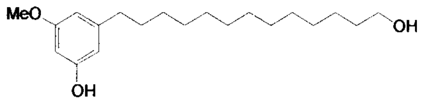


(**81**) 1,3-dihydroxy-5-(2'-hydroxy-8'-oxotridecyl)benzene [209]

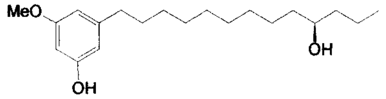
Fig. (4) cont.



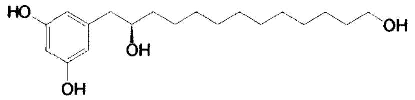

(**82**) 1,3-dihydroxy-5-(2'-acetoxy-8'-hydroxytridecyl)benzene [209]



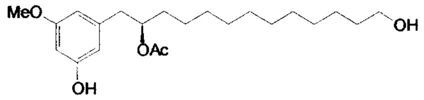

(**83**) 1-hydroxy-3-methoxy-5-(13'-hydroxytridecyl)benzene [209]



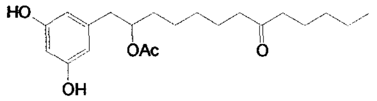

*(**84**) (10'*R*)-1-hydroxy-3-methoxy-5-(10'-hydroxytridecyl)benzene [170]



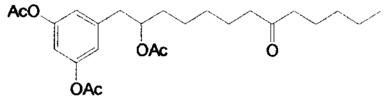

(**85**) (2'*R*)-1,3-dihydroxy-5-(2',13'-dihydroxytridecyl)benzene [170]



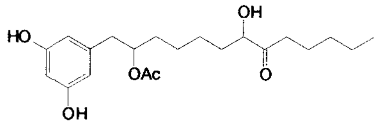

*(**86**) (2'*R*)-1-hydroxy-3-methoxy-5-(2'-acetoxy-13'-hydroxytridecyl)benzene [170]




*(**87**) 1,3-dihydroxy-5-(2'-acetoxy-8'-oxytridecyl)benzene [172]

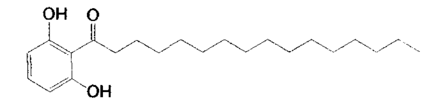



*(**88**) 1,3-diacetoxy-5-(2'-acetoxy-8'-oxotridecyl)benzene [172]

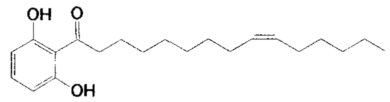



*(**89**) 1,3-dihydroxy-5-(2'-acetoxy-7'-hydroxy-8'-oxotridecyl)benzene [172]

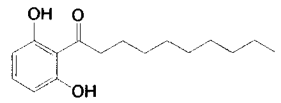
Fig. (4) cont.



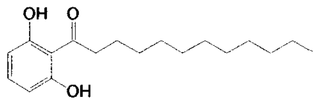

(**90**) 1,3-dihydroxy-2-(1'-oxohexadecyl)benzene [159]



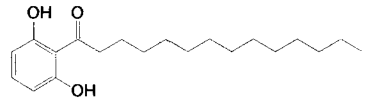

(**91**) 1,3-dihydroxy-2-(1'-oxopentadec-9'*Z*-enyl)benzene [35]



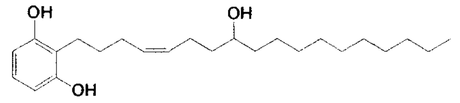

(**92**) 1,3-dihydroxy-2-(1'-oxodecyl)benzene [35]



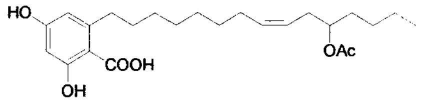

(**93**) 1,3-dihydroxy-2-(1'-oxododecyl)benzene [35]



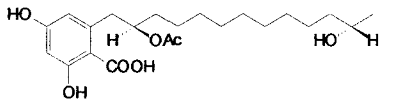

(**94**) 1,3-dihydroxy-2-(1'-oxotetradecyl)benzene [35]



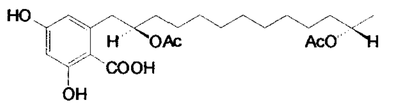

(**95**) 1,3-dihydroxy-2-(8-hydroxyoctadec-4*Z*-enyl)benzene [35]




(**96**) 2,4-dihydroxy-6-(11'-acetoxypentadec-8*Z*-enyl)benzoic acid [169]

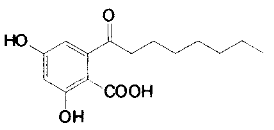



(**97**) (2'*R*),(12'*S*)-2,4-dihydroxy-6-(2-acetoxy-12-hydroxytridecyl)benzoic acid [173]

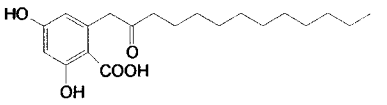



(**98**) (2'*R*),(12'*S*)-2,4-dihydroxy-6-(2,12-diacetoxytridecyl)benzoic acid [173]

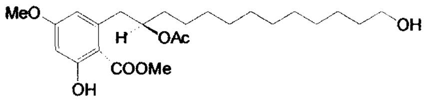
Fig. (4) cont.



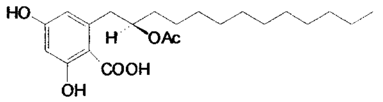
(**99**) 2,4-dihydroxy-6-(1-oxooctyl)benzoic acid [195]



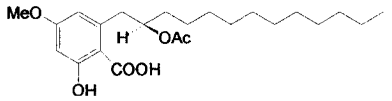
(**100**) 2,4-dihydroxy-6-(2-oxotridecyl)benzoic acid [170]



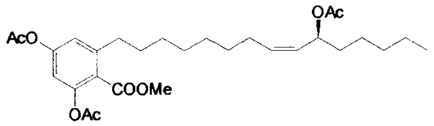
*(**101**) (2'*R*)-2,4-dihydroxy-6-methoxy-(2'-acetoxy-13'-hydroxytridecyl)benzoic acid [170]



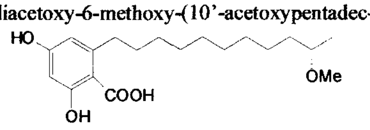
*(**102**) (2'*R*)-2,4-dihydroxy-6-(2'-acetoxytridecyl)benzoic acid [170]



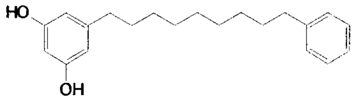
*(**103**) (2'*R*)-2-hydroxy-4-methoxy -6-(2'-acetoxytridecyl)benzoic acid [170]



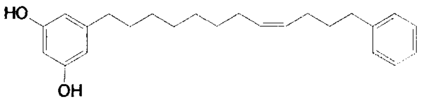
(**104**) Methyl (10'*R*)-1,3-diacetoxy-6-methoxy-(10'-acetoxypentadec-8*Z*-enyl)benzoate [175]



(**105**) (10'*S*)-1,3-dihydroxy-6-(10'-methoxyundecyl)benzoic acid [171]

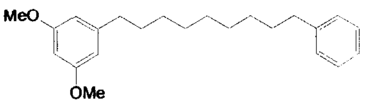


(**106**) 1,3-dihydroxy-5-(9-phenylnonyl)benzene [86,87]

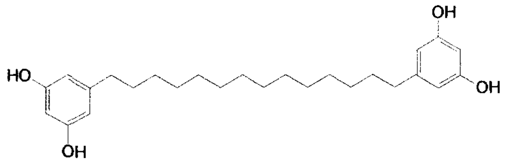


(**107**) 1,3-dihydroxy-5-(12-phenyldodec-8*Z*-enyl)benzene [158]

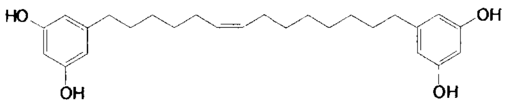
Fig. (4) cont.



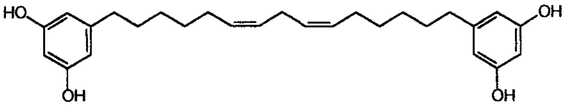

(**108**) 1,3-dimethoxy-5-(9-phenylnonyl)benzene [86,87]



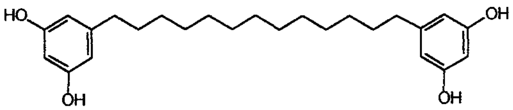

(**109**) 1,3-dihydroxy-5-[14-(3',5'-dihydroxyphenyl)tetradecyl]benzene [147,210]



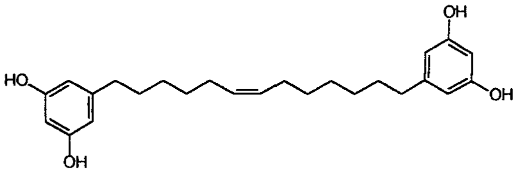

(**110**) 1,3-dihydroxy-5-[14-(3',5'-dihydoxyphenyl)tetradec-6*Z*-enyl]benzene [138,147]




(**111**) 1,3-dihydroxy-5-[15-(3',5'-dihydroxyphenyl)pentadec-6Z,9Z-dienyl]benzene [144]

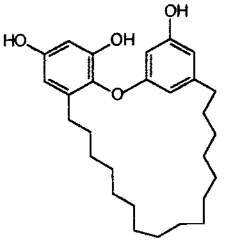



(**112**) 1,3-dihydroxy-5-{15-(3',5'-dihydroxyphenyl)tridecyl]benzene [144]

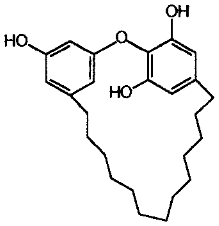



(**113**) 1,3-dihydroxy-5-[15-(3',5'-dihydroxyphenyl)tridec-6Z-enyl]benzene [144]

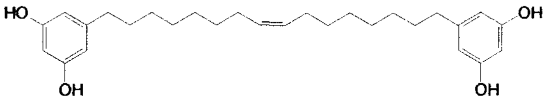
Fig. (4) cont.



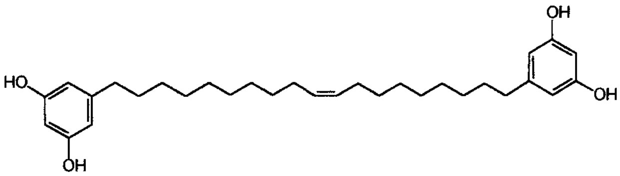

(**114**) 5,5'-hexadecamethylene-1,1',3-trihydroxy-4,3'-diphenylether [144]



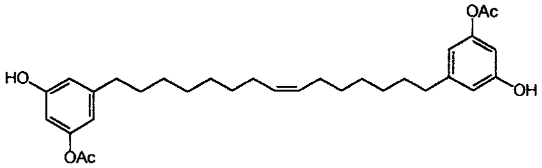

(**115**) 5,5'-tridecamethylene-1,1',3-trihydroxy-4,3'-diphenylether [144]



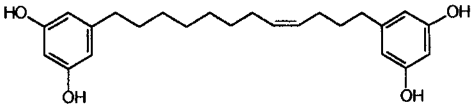

(**116**) 1,3-dihydroxy-5-[16-(3',5'-dihydroxyphenyl)hexadec-8*Z*-enyl]benzene [147,156]




(**117**) [156]

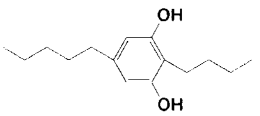



(**118**) [156]

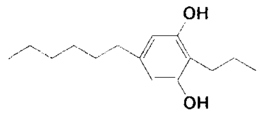



(**119**) [156]

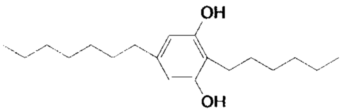
Fig. (4) cont.



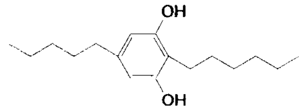
**(120)** 2-butyl-1,3-dihydroxy-5-pentylbenzene [194]



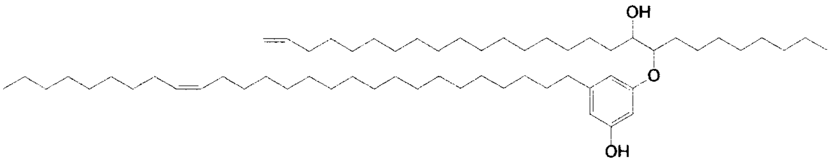
**(121)** 1,3-dihydroxy-5-pentyl-2-propylbenzene [111]



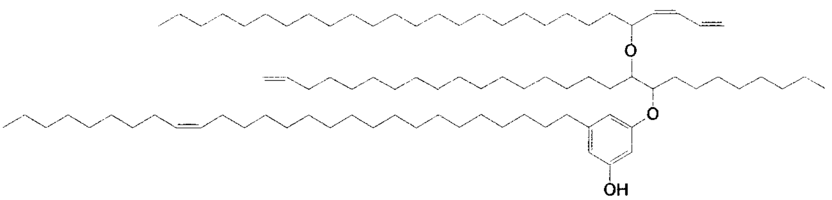
**(122)** 1,3-dihydroxy-5-heptyl-2-hexylbenzene [108]



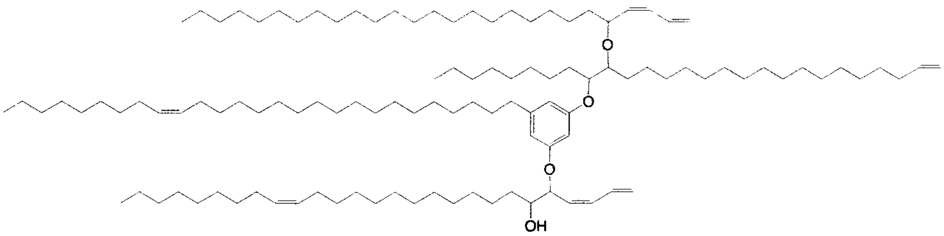
**(123)** 1,3-dihydroxy-2-hexyl-5-pentylbenzene [108]



**(124)** 5-(heptacosa-18'Z-enyl)-3-hydroxy-1-(18"-hydroxy-1"-enyl-19"-oxy)benzene [191]



**(125)** 1-[18"-(1'",3'"-dienylheptacosa-5'"-oxy)heptacosyl-1"-enyl-19"-oxy]-5-(heptacosa-18'-enyl)-3-hydroxybenzene [191]



**(126)** 5-[(heptacosa-1'",3'"-dienyl-5'"-oxy)-18"-oxyheptacosa-1"-enyl-19"-oxy]-1-(heptacosa-18Z-enyl)-3-(6'-hydroxyheptacosa-1',3',18'-trienyl-5'-oxy)benzene [191]

The compounds comprise homologous 1,3-dihydroxy-5-alkylbenzenes from $C_5$ to $C_{29}$, structurally related monoenes, dienes and trienes, a number of nuclear C-methyl, dimethyl and several OH-substituted derivatives all possessing the 1,3-dihydroxy-5-alkyl grouping. A range of chiral compounds of this structural type but substituted in the side-chain with acetoxy, hydroxy, methoxy groups, and others with phenyl, 3,5-dihydroxyphenyl or carbonyl groups is depicted*. Homologous orsellinic acids, essentially, 2,4,6-substituted benzoic acids, with similar structural features to all the previous compounds are shown. A number of 2-alkylresorcinols and 2,5-dialkylresorcinols, although, remarkably no 4-alkylresorcinols, are depicted and finally, certain complex interaction products of very long chain 5-alkyl-1,3-dihydroxybenzenes with alkane-1,2-diols and alkenols. In most cases the phenolic hydroxylic groups remain unmodified.

It is worth noting that in many cases some of the resorcinolic lipids present in biological material still remain structurally uncharacterised. For instance, chromatographic analysis of acetone extracts from cereal grains shows the presence of at least 4 other components that probably belong to the group of resorcinolic lipids. Two of them have been identified as 5-(2-oxoalkyl) resorcinol and 5-(2-oxoalkenyl) resorcinols [178] and the another as a 5-(2-hydroxyalkyl) resorcinol [181].

The Leguminaceous plants, mostly of the genus *Ononis* have been recently demonstrated to be a source of numerous resorcinolic lipids. They include both ring and chain-modified derivatives of various length of the aliphatic side chain. The modifications include occurrence of free or modified hydroxy or/and keto substituents in the 5-alkyl chain and of the ring-attached hydroxylic groups to form 1,3-dihydroxy-2-alkyl and 1,3-dihydroxy-2,5-dialkylbenzenes - (Fig 4, structures **90-95** and **120-123**, respectively). The presence of unmodified 5-n-alk(en)ylresorcinols have been also demonstrated in other leguminaceous plants, including the common pea [176,211]. A most frequent modification in 5-alkylresorcinols is the occurrence of a carboxylic group in the ring resulting in an alkylresorcinolic acid or orsellinic acid derivatives also known as merulinic acid (2,4 dihydroxy-6-alkylbenzoic acid) (**56-59**).

* It would appear that the number of compounds described in refs. 170-172 are artefacts arising from acetylation/methylation during isolation. They are marked with an asterisk (*) in those cases.

Bis-alkylresorcinols (Fig 4, structures **109-113** and **116-119**) have been demonstrated in plant material [138,144,147,156] together with their cyclic derivatives (e.g., Fig 4, structures **114**, **115**). From Bolivian algae Metzger has isolated other oligomeric derivatives [191] (Fig 4, structures **124-126**).

## SEPARATION OF PHENOLIC LIPIDS

Prior to structural elucidation and possible eventual synthesis, the isolation of component phenolic lipids in a pure state is essential. The cold methods of thin layer chromatography (TLC), column chromatography (CC), flash chromatography, high performance liquid chromatography (HPLC) and hot methods (GC), often after derivatisation, are well established. Argentation versions of these separatory methods are less common but are desirable for the rapid separation of unsaturated constituents.

Thus reversed phase argentation HPLC has been employed for gingolic acids in *Ginkgo biloba* [212,213]. For this same species, other chromatographic techniques have been applied [214-216]. Catechols are sensitive to the argentation method and in this group HPLC and GC procedures have proved beneficial particularly, for example, with the latter approach [217]. Bioassay-directed fractionation has been found valuable with 5-alkylresorcinols from the leaves of *Oncostemon bojerianum* [156] and for ardisiaquinones (Myrsinaceae species) [218].

For biological evaluation larger quantities of component phenols and their constituents may be required and where different components such as alkylresorcinols and anacardic acids co-occur, an initial solvent separation may prove desirable. Mild extraction methods, such as supercritical fluid extraction with carbon dioxide, [1,219,220], followed by phase separation [221], preparative TLC [222], or column chromatography [223] may be valuable for complex mixtures.

## SYNTHESIS OF PHENOLIC LIPIDS

Synthesis has often followed structural elucidation of a phenolic lipid to complete the characterisation process. However it may also become of greater significance in the study of biological properties, particularly in the involvement of structure/activity relationships.

Biological investigations have indicated that the level of unsaturation and the geometric configuration of double bonds are salient factors. Thus the regiospecificity of the synthetic method adopted becomes important and the assessment of purity of the product notably by spectroscopy, HPLC and argentation TLC are significant criteria. Synthetic methods for obtaining phenolic lipids have been reviewed [1-3,5,11,131]. In this account, emphasis has been placed on methods more particularly developed in the last decade.

Methods of synthesis of saturated, monoene, diene and trienes components by five different strategies are discussed based upon:

(1) The progresive attachment of synthons to form the unsubstituted side-chain of an (OH) protected phenol bearing a CHO, $CO_2R$, $CO_2H$ or COCl group, by nucleophilic addition reactions with organometallic reagents (alkyl and alkynyllithiums).

(2) The alkylation of an activated Me group in an aryl moiety with a preassembled 1-haloalk(en)yl compound to form the side-chain.

(3) The use of acyclic precursors by Claisen cyclisation or Diels-Alder addition reactions.

(4) Reactions of methoxybenzenes or those bearing a fluoro substituent with lithium, an alkyllithium. or a bromo substituent with a Grignard reagent.

(5) Formation of oxo, hydroxy substituted saturated side-chains by reactions of methoxycinnamic or phenylacetic acids.

## (1) **Synthesis by successive attachment of synthons**

The synthesis of phenolic lipids of all types bearing saturated side-chains presents few problems and has been approached by the methodologies of classical organic chemistry [1,2,11,131].

With unsaturated phenolic lipids, the first syntheses of Fig (1)-**1**, cardanol (8Z)-monoene and Fig (1)-**3**, (8Z,11Z)-cardanol diene used the synthon sequences (MeOArCHO → $MeOArC_7$ → $MeOArC_{15}$) and (MeOArCHO → $MeOArC_7$ → $MeOArC_{10}$ → $MeOArC_{15}$ ) respectively, by acetylenic routes [224] [19], which gave in each case the phenolic lipid. The resorcinolic lipids, Fig (4)-**18**, cardol monoene and Fig (4)-**20**, diene were obtained by similar routes [225]. A recent synthesis of climacostol [113] has been effected by the reaction of HO-protected 3,5-dihydroxybenzaldehyde with 1-lithiooctyne, followed by protection

of the alkyne bond, hydrogenolysis and selective formation of the 3(Z)-alkene

Fig (3)-**2**, Urushiol (8Z)-monoene and Fig (3)-**3**, (8Z,11Z)-diene were synthesised in a comparable synthon sequences from 2,3-dimethoxybenzaldehyde [226,227]

However, on account of the unavailability of the corresponding aldehyde, anacardic acid (8Z)-monoene Fig (2)-**2** and Fig (2)-**3**, the (8Z,11Z)-diene were obtained by the alkylation route as described in [2].

These general methods may also be applied to unsaturated $C_{17}$ compounds and other structural isomers.

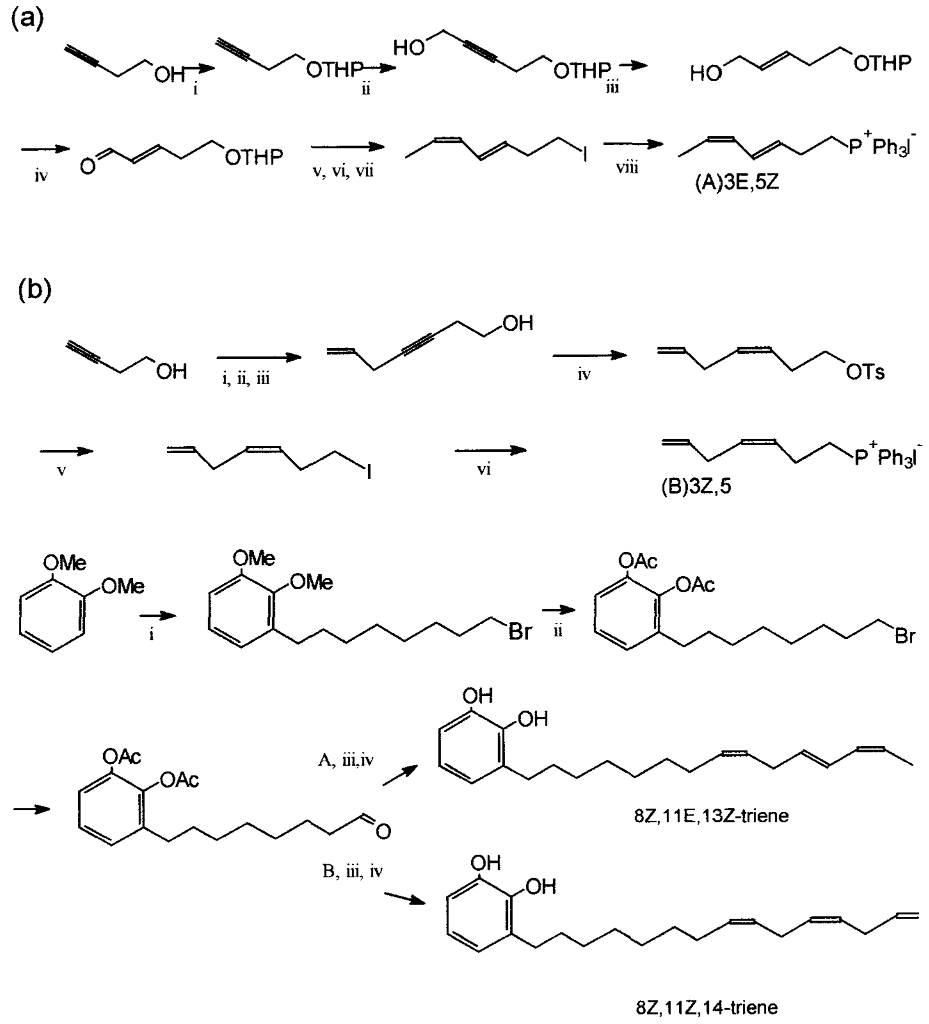
For Fig (1)-**4**, cardanol and Fig (4)-**21**, cardol (8Z,11Z,14)-trienes, with modified $C_5$ synthons (pent-1-yn-4-ene, and pent-1,4-diyne), the method was less satisfactory [2] due to lack of selectivity during final catalytic hydrogenation.

With 3,5-dimethoxybenzoyl chloride used in the monoacylation of cyclohexanone to produce finally the same $(MeO)_2ArC_7$ synthon, an alternative synthesis of cardol 8(Z)-monene was developed [138]. Fig( 4)-**49**, Persoonol dimethyl ether (5-[(Z)-undec-3-enyl]resorcinol dimethyl ether) was synthesised from 3,5-dimethoxybenzyl alcohol [149]. This same reactant has been employed for a range of 5-substituted resorcinols although its application to unsaturated phenolic lipids was not discussed [228]. Acetylenic methods have proved valuable for deriving phenolic lipidic double bonds in the Z-configurations and with advances in boration methodology [229] the corresponding E-isomers are accessible.

The Wittig reaction approach provides an alternative route to the synthesis of the unsaturated phenolic lipids in urushiol in *Rhus vernicifera*, which contains three $C_{15}$ isomeric trienes, the major constituent the semi-conjugated Fig (3)-**4**, 8(Z),11(E),13(Z), (55.4%), the trace corresponding 13(E) isomer (1.7%) and the non-conjugated Fig (3)-**5**, 8(Z),11(E),14 (7.4%) which is also the chief component in urushiol from *Rhus toxicodendron.* Scheme 1 shows the routes to the first and last constituents [230-233].

The same heptadienyl phosphonium phosphonium salt (B) has been used for a synthesis of the germination stimulant occurring in *Sorghum bicolor,* for the seeds of the parasitic *Striga asiatica,* namely (Fig (4)-**66**) 5-methoxy-3-[(Z,Z)-pentadeca-8,11,14-trienyl]-1,2,4-trihydroxybenzene [208] by a Wittig procedure.

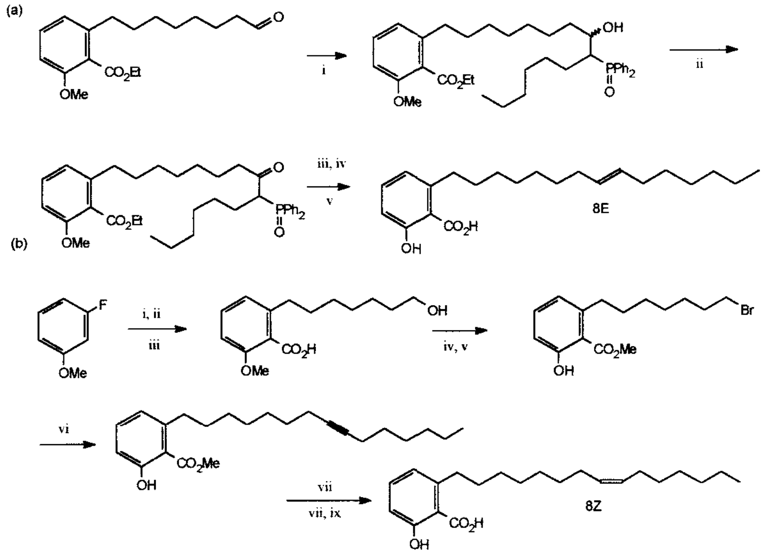
Thitsiols. (3,4-dihydroxy isomers of urushiol), particulaely the triene Fig (3)-**6**, have been synthesised [185].




Scheme 1. Synthesis of (15:3)-8(Z),11(E),13(Z) and of 8(Z),11(Z),14 urushiols

Reagents: (a) (i) DHP, $H^+$, (ii) EtMgBr, HCHO, (iii) $LiAlH_4$ , (iv) PDC, (v) $EtP^+Ph_3 Br^-$ ,BuLi , (vi) MeOH, $H^+$ , (vii) $PPh_3$, $CBr_4$; KI, $Me_2CO$, (viii) $PPh_3$, MeCN, (b) (iii), 3-(8-oxo-1-octyl)catechol) diacetate, A, BuLi , (iv) $LiAlH_4$, t-BuOH .
(b) (i) 4-TSA, DHP, (ii) EtMgBr, CuCl, $CH_2$=$CHCH_2Br$, (iii) 4-TSA, MeOH, (iv) $Pd/BaSO_4/H_2$ ,py.; 4-TsCl, py., (v) NaI, $Me_2CO$, (vi) $PPh_3$ , (b) (iii) 3-(8-oxo-1-octyl)catechol) diacetate, B, BuLi, (iv) LiAlH, t-BuOH.

The isomer of Fig (2)-**2**, anacardic acid 8(E)-monoene has been synthesised by a *trans* elimination reaction as depicted in Scheme 2 [234], which also depicts the route used for the (Z)- isomer, ginkgolic acid. [235].

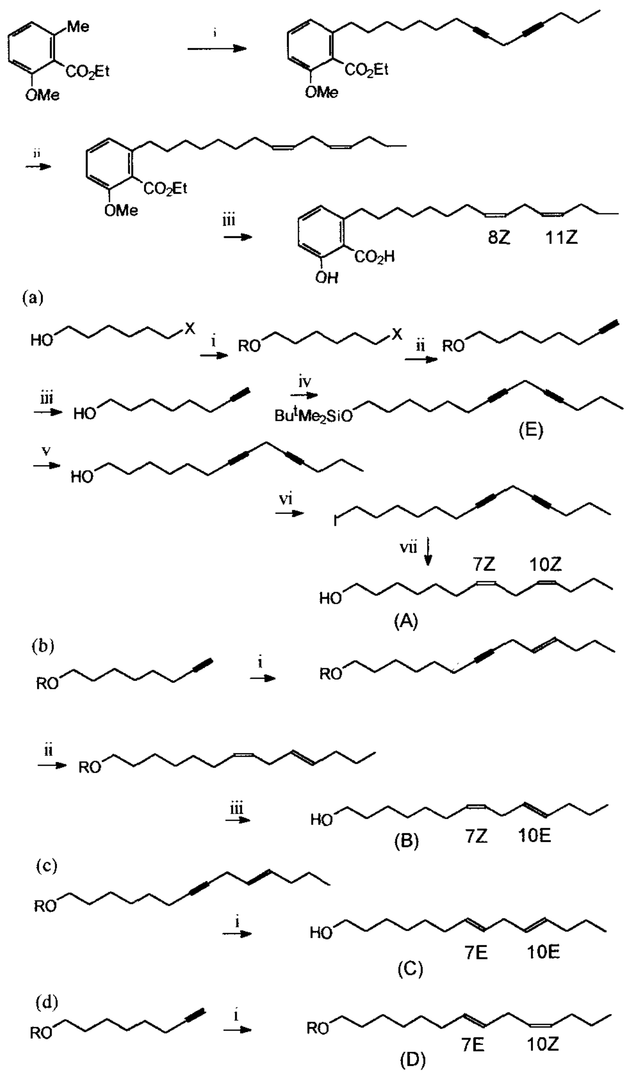



Scheme 2. Synthesis of anacardic acid 8(E)- and (Z)-monoenes

Reagents: (a) ( i) $C_7H_{15}P(O)Ph_2$ , BuLi, (ii) $HCO_2H,H_2O_2$, (iii) $NaBH_4$, (iv) threo sepn., (v) NaH, DMF.
(b) (i) $Li(CH_2)_7OTHP$, (ii) $CO_2$, (iii) $H^+$, (iv)$BBr_3$, -78°; $H^+$ (v) $CH_2N_2$ ,
(vi) Li 1-octyne, HMPT, THF, (vii) $Pd/BaSO_4/H_2$, quin., (viii) $HO^-$ , (ix) $H^+$.

## (2) Synthesis by the alkylation of an activated methyl group with a preassembled ω-haloalkane, alkene or alkyne

The initial application to the synthesis of anacardic acid-8Z,11Z-diene, used the synthons, $ArC_1$ → $ArC_7$ → $ArC_{10}$ →$ArC_{15}$ [114,236]. Subsequently, this approach to the synthesis of $C_{15}$ phenolic lipids consisted in the route $ArC_1$ → $ArC_{15}$. It has proved generally convenient for the synthesis of the unsaturated anacardic acids. From the methodological view it is also an aspect of the use of acyclic intermediates discussed in the next section since the starting material ethyl 2-methoxy-6-methylbenzoate is prepared from ethyl acetoacetate.

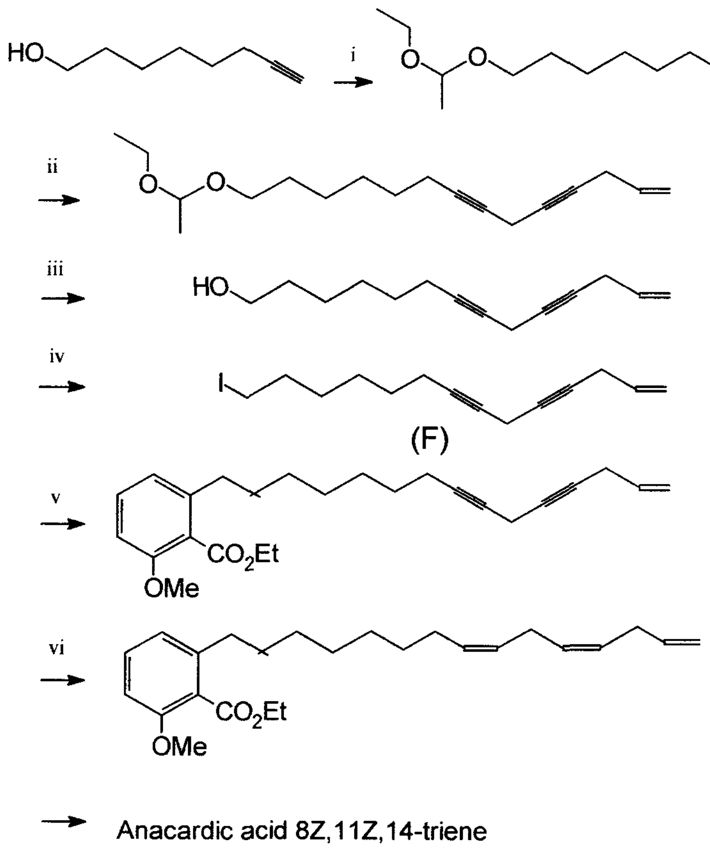



Scheme 3. Synthesis of anacardic acid 8(Z),11(Z)-diene and $C_{14}$ reactant stereoisomers

Reagents: (i) $LiNPr_2$, HMPT, THF, (a) E, -70°- 25°C, (ii) Pd/$BaSO_4$,$H_2$,EtOH, quin..(iii) $LiSBu^t$, DMF; $HO^-$.

(a) (i) $CH_2$=CHOEt, 4-TSA, (ii) LiC≡CH, $NH_3$, THF, HMPT, (iii) MeOH, HCl, (iv)$Bu^tMe_2SiCl$, DMF, imidazole; EtMgBr, THF, CuCl, $BrCH_2C≡CC_3H_7$, (v) $Bu_4N^+F^-$, THF, (vi) $Me_3SiCl$, py.; NaI, MeCN, (vii) Pd/$BaSO_4$, $H_2$, quin. (b) EtMgBr, THF, CuCl, (*E*)-$BrCH_2CH$=$CHC_3H_7$, (ii) Pd/$BaSO_4$, $H_2$, quin., (iii) MeOH, HCl. (c) Na/$NH_3$, THF, HMPT; MeOH, HCl. (d) (i) $RO(CH_2)_6C≡CH$, EtMgBr, CuCl, THF, (*Z*)-$BrCH_2CH$=$CHC_3H_7$; Na/$NH_3$; MeOH, HCl.

Scheme 3 depicts, for anacardic acid diene, the synthesis of H, by the alkylation method with a $C_{14}$ halide and the synthesis of ω-hydroxy $C_{14}$ the side-chains for the 8,11-diene and its stereoisomers namely (A) 7Z,10Z, (B) 7Z,10E, (C) 7E,10E and (D) 7E,10Z.. The 1-iodo derivatives of each were obtained as for route (a). This methodology has been applied to the synthesis of Fig (2)-**3**, anacardic acid 8(Z),11(Z),14-triene as shown in Scheme 4. Strategies for obtaining side-chains for the stereoisomers of the triene have been described [2,114].




Scheme 4. Synthesis of Anacardic acid 8Z),11(Z),14-triene

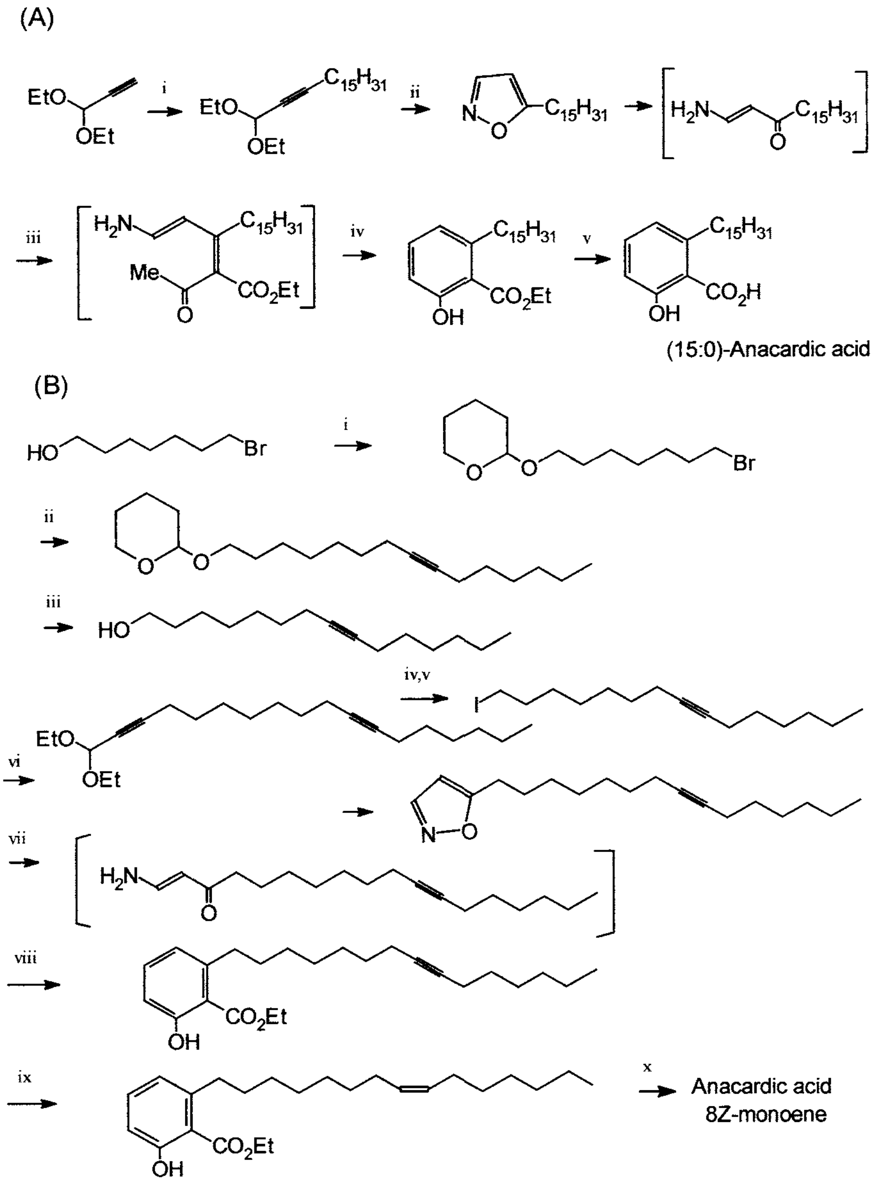
Reagents: (i) 4-TSA, $CH_2$=CHOEt, (ii) EtMgBr, THF, CuCl, $BrCH_2C{\equiv}CCH_2CH{=}CH_2$, (iii) MeOH,HCl, (iv) $Me_3SiCl$, py. MeCN, NaI, (v) $LiNPr_2$, THF, HMPT, $MeC_6H_3(OMe)(CO_2Et)$, F, (vi) $(iC_5H_{11})BH$, THF; $HO^-$, $LiSBu^t$.

## (3) Synthesis from acyclic precursors

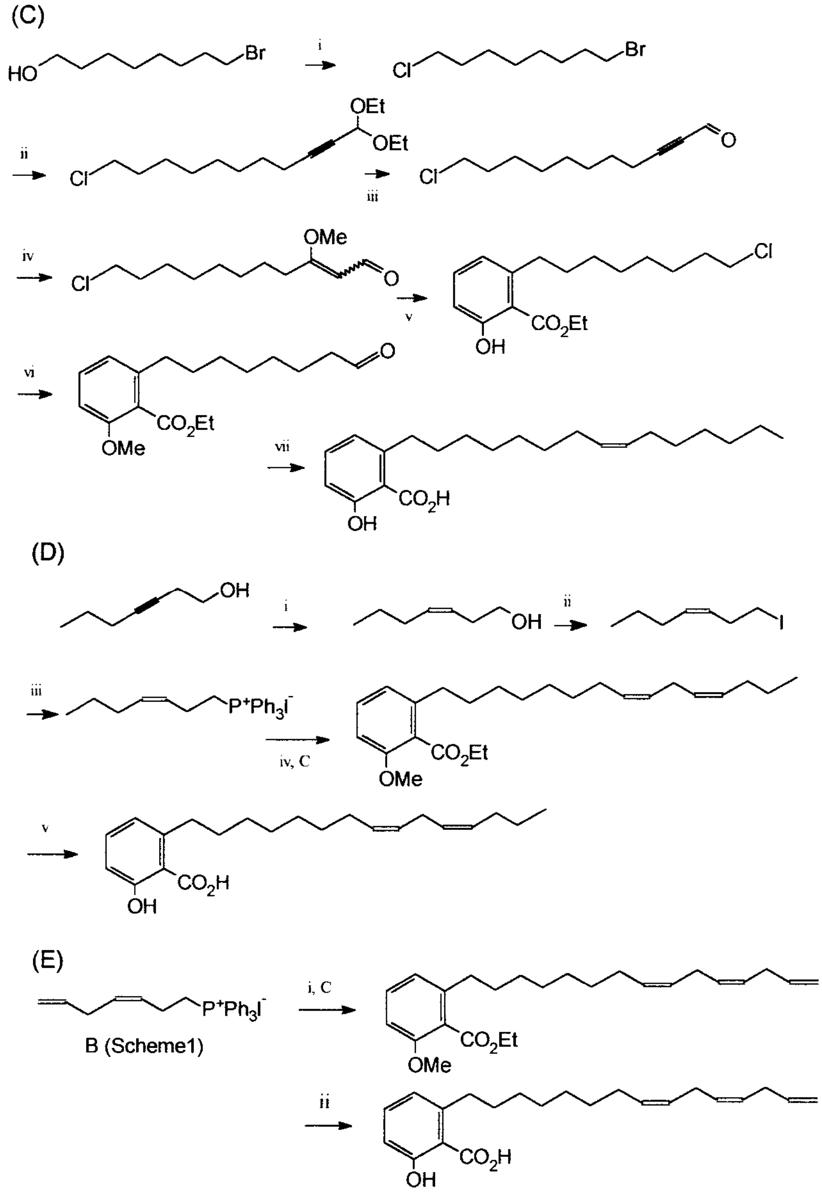
The first synthesis modelled on biomimetic lines was directed to obtaining anacardic acids by way of polyketides [237] and later to a (17:1)-orsellinic acid [43]. A less complicated approach based on the Michael addition of ethyl acetoacetate and ethyl octadec-2-enoate, has led to a $C_{15}$ orsellinic acid, Fig (4)-**56**, 2,4-dihydroxy-6n-pentadecylbenzoic, considered to be the biogenetic precursor of the cashew phenols [238], notably cardol, by decarboxylation. The use of bromine at the aromatisation stage in this synthesis precluded the extension of the method to components with unsaturated side-chains, although bromination with copper(ii)bromide and thermal debromination offers an alternative procedure. In a more recent approach, by the use of an oxazole intermediate and its addition to ethyl acetoacetate, (15:0) and (15:1) anacardic acid have been obtained [239] as shown in Scheme 5a, b. The 8(Z),11(Z)-diene and 8(Z),11(Z),14-triene have also been synthesised [240] by way of ethyl 6-(7-formylheptyl)-2-methoxybenzoate (C), prepared from acyclic sources, rather than, as in previous work, by semi-synthesis from the ozonisation of urushiol.

Wittig reactions then afforded the unsaturated phenolic lipids after demethylation and alkaline hydrolysis as depicted in Schemes 5b and c.

Anacardic acid 8(Z),11(Z)-diene has been prepared by the Diels-Alder addition of 1-methoxycyclohexa-1,4-diene to an homologous propiolic ester [241] with elimination of ethylene. The methoxydiene intermediate was prepared by the Birch reduction of methoxybenzene and this approach represents a link between the use of an aromatic and a derived acyclic precursor.




Schemes 5. Syntheses of (15:0), (15:1), (15:2) and (15:3)-anacardic acids.




Schemes 5 cont. Syntheses of (15:0), (15:1), (15:2) and (15:3)-anacardic acids.

Reagents: (A) (i) $C_{15}H_{31}Br$, BuLi, HMPT, THF, (ii) $NH_2OH.HCl$, EtOH; $Fe_2(CO)_9$, (iii) $MeCOCH_2CO_2Et$, NaH, BuLi, $BF_3Et_2O$, (iv) $Ca(OAc)_2$, (v) $HO^-$. (B) (i) TSA, DHP, (ii) $C_6H_{13}C{\equiv}CH$ , BuLi, HMPA, THF, (iii) EtOH,HCl , (iv) $SOCl_2$, py., (v) NaI, MeCOEt,

(vi) $HC{\equiv}CCH(OEt)_2$, BuLi, HMPA, THF (vii) $NH_2OH.HCl$, EtOH; $Fe_2(CO)_9$, (viii) $MeCOCH_2CO_2Et$, NaH, BuLi, $BF_3Et_2O$; $Ca(OAc)_2$, (ix) $Pd/CaCO_3$, $H_2$, $C_6H_{14}$, (x) $HO^-$. (C) (i) $SOCl_2$, py., (ii) $HC{\equiv}CCH(OEt)_2$, BuLi, HMPA,THF, (iii) $H_3PO_4$ , $H_2O$, (iv) MeONa, MeOH, (v) $MeCOCH_2CO_2Et$, NaH,BuLi, THF; KF, $MeC_6H_5$, (vi) MeI, $Ag_2O$; NaI, $Me_2CO$; $NaHCO_3$, DMSO, (vii) $Me(CH_2)_6P^+Ph_3I^-$, BuLi; $BBr_3$; $HO^-$, EtOH. (D) (i) $Pd/CaCO_3$, $H_2$, quin., (ii) $PPh_3$, $I_2$, imidazole, MeCN, (iii) $PPh_3$ , MeCN, (iv) BuLi, THF, C, (v) $BBr_3$, $CH_2Cl_2$ ; $HO^-$. (E) (i) $CH_2{=}CHCH_2CH{=}CH(CH_2)_2P^+Ph_3I^-$ (B, Scheme 1) BuLi, THF, (ii) $BBr_3$ , $CH_2Cl_2$ ; $HO^-$.

### (4) Synthesis from methoxybenzene(s) or fluoro or bromo derivatives by reaction with organometallic reagents.

Anacardic 8(Z)-monoene (ginkgolic acid) has been synthesised from 2- or 3-methoxyfluorobenzene by the sequence, MeOArF → $MeOArC_7$ → $HOArC_{15}$ [242] (as shown in Scheme 2b) and orsellinic acids with 3,5-dimethoxyfluorobenzene [235]

Urushiol analogues with $C_{18}$ alkenyl side chains have been synthesised from 3-bromobenzene and 2,3-acetonylidenebromobenzene by cross coupling with Grignard reagents derived from $C_{18}$ unsaturated halides [217,243].

3-(ω-phenylalkyl)catechols (Fig (3)-7) from the Burmese lac tree) have been obtained from lithiation of 2-methoxmethoxyanisole and reaction with ω-phenyldodecanal followed by hydrogenolysis [244].

Saturated cardols have been synthesised from 3,5-dimethoxyphenol through the use of the derived triflate by cross-coupling reaction with a borane component in the presence of $PdCl_2$/NaOMe [210].

### (5) Formation of oxo and hydroxy substituted saturated side-chains from methoxycinnamic and methoxyphenylacetic acids

An increasing number of phenolic lipids with hydroxy or oxo substituted side-chains has been isolated in recent years. Thus, the $C_{17}$, 5-(2-oxoalkylresorcinol, Fig (4)-**70**, found in *Cereale secale,* has been synthesised from 3,5-dimethoxyphenylacetic acid [181].

The (S)-(+)-gingerols, 4-hydroxy-3-methoxy-(5S-hydroxy-3-oxoalkyl)benzenes, comprise a group of homologous $C_{10}$ , $C_{12}$ and $C_{14}$ compounds. The last (Fig (3)-**8**), has been synthesised from 4-hydroxy-3-methoxycinamic acid [245].

# BIOLOGICAL PROPERTIES OF PHENOLIC LIPIDS

The properties of phenolic lipids have tended to be dominated by technological aspects and it is only comparatively recently that potential biological usefulness has come to the fore. For example, products derived from the *Anacardiacae occidentale*, notably cardanol obtained by semi-synthesis through thermal decarboxylation were all directed to polymeric and technical applications and the vast industrial literature [246] contains only two references to insecticidal uses of chlorinated cardanol [247].

Nevertheless, by contrast, anacardic acid, the first phenolic lipid to be isolated [248], although its structure was not determined until much later [11], was examined during the thirties of the last century for potential antibacterial activity. The biological value of cardols particularly of bilobol from *Ginkgo biloba* (Ginkgoaceae) appears to have been recognised at an early stage [3,11] and in general, a great deal more work has been devoted to the biological properties of resorcinolic lipids.

This section describes the biological properties of the range of the monohydric and dihydricphenols and the phenolic acids depicted in Figs. 1, 2 and 4. At this juncture the properties of urushiols have not been included but will be the subject of a future summary.

## Biological Properties of Cardanols

Historically the main interest in cardanol, in the form of the semi-synthetic, technical cashew nutshell liquid, obtained by thermal decarboxylation of natural cashew nutshell liquid, has been in a wide range of technological applications such as in friction dusts and in polymer chemistry many of which have been described in a number of reviews [1,2,11]. Remarkably, very new biological applications have been found for this versatile raw material.

Apart from cardanol (Fig. 1-**1**,**2**,**4**, and **5**) which collectively is a minor natural component in *Anacardium occidentale*, the first 3-substituted monohydric phenols isolated were, ginkgol (identical with cardanol monene), (depicted in Fig.1-**2**), from *Ginkgo biloba,* and camnospermanol, (Fig.1-**6**), from *Camnospermum auriculata.* More recently a number of 4-substituted monohydric phenols, (depicted in Fig.1), have been obtained from natural sources including, elenic acid, *R*-2,4-dimethyl-22-(4-hydroxyphenyl)-docos-3(E)-enoic acid, **7**, navenone,

**9,** promalabaricone **8**, prepromalabaricone, **10**, plakinidone, **11**, the ester, flexirubin, **12,** from a gliding bacterium, and habaneriol, **14**. Phenolic glycolipids, for example, ω-[(4-glycosyloxy)phenyl]phthiocerol, and **13**, both from *Mycobacterium spp.* are important relatives of the phenolic lipids [249].

Elenic acid, **7**, was isolated from an Indonesian sponge *Plakinastrella* sp. [25]. It is an enzyme inhibitor of DNA topoisomerase II with an $IC_{50}$ of 0.1μg/ml [25] and also a potent inhibitor of calf DNA polymerase α and rat DNA polymerases β [250]. Elenic acid was found not to bind to DNA directly and did not affect the activities of plant DNA polymerases I and II, prokaryotic DNA polymerases such as *Escherichia coli* DNA polymerase I or other DNA metabolic enzymes, for example, HIV reverse transcriptase, T7 RNA polymerase and bovine deoxyribonuclease I. Elenic acid did not prevent the growth of NUGC-3 cancer cells and the $LD_{50}$ was 22.5μM.

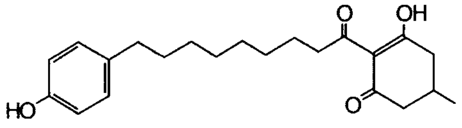
Monohydric phenols with alkyl chains are rare amongst marine natural products and navenone [27], (Fig. 1-**9**), is a component of the trail pheromone of the mollusc, *Navanax inermis*, while plakinidone, (Fig. 1-**11**), was isolated from the Caribbean sponge, *Plakortis angulospiculatus* (Plakinidae) [26].

Habenariol, **14** is another metabolic compound associated with an aquatic environment. It is an antifeedant metabolite from the aquatic orchid, *Habenaria repens* which protects against the common freshwater crayfish, *Procambarus clarkii.* [21]. Thus, the aquatic orchid becomes a low preference food for the crayfish.

Other investigators have examined the potential antioxidant properties of habenariol [251] particularly to inhibit copper-induced lipid peroxidationof human low density lipoprotein (LDL). However, in both kinetic and end-point spectrophotometric assays, it was found to be less effective than α-tocopherol.

A new C-allyl cardanol derivative formed by Claisen rearrangement of an allyl ether namely, 2-allyl-3n-pentadecyl-6-tert-butylphenol had comparable antioxidant properties to the well known compounds DBHA and BHT, respectively 2,6-di-tert-butyl-4-methoxyphenol and 2,6-di-tert-butylphenol [252]. The 2-allyl compound is related in properties to the precursor 2-tert-butyl-5n-pentadecylphenol (see, e.g. p. 542 in ref.[1] and [253] and to the 'hindered' phenols [254].

Prepromalabaricone B, (Fig.1-**10**), and promalabaricone B, (Fig.1-**8**), which represents a cyclised form resulting from dehydration, together with giganteones, **14** and **15,** (Fig.3), have been isolated from the fruits of *Myristica gigantea* (Myrisicaceae) [19,20]. The compounds exhibited cytotoxic activity on human nasopharynx KB cells with 81% inhibition at 10μg/ml.




promalabaricone B

The skin irritancy of cardanol monoene isolated from the fruits of the spice pink pepper, *Schinus terebinthifolius*, has been examined. However, it was supposed that the irritative effect, which was manifested after a long latency period, was also contributed to by ingredients of the essential oil, for example, by phellandrene and delta-3 carene [255]. Accordingly, it was indicated that experimental tests with animals were necessary 'before the spice can be forbidden for sale'.

## Biological Properties of Anacardic Acids

### *Antibacterial, antimicrobial, antifungal and antialgal activity*

The antibacterial, antimicrobial properties of anacardic acid itself from *Anacardium occidentale* (Fig.2-1,2,3 and 4) are legendary [11]. In recent years other botanical sources such as *Ginkgo biloba* have been examined for antimicrobial activity [256]. More recent studies have focussed on the action of anacardic acids on specific organisms such as in the use of formulated products against *Propionibacterium acnes* [257]. The bactericidal effect of anacardic acid and its potentiation towards *Streptococcus mutans* [258] and of anacardic acid with totarol towards methicillin-resistant *Staphylococcus aureus* (MRSA) has been investigated [259-261]. Anacardic acid was found to be a specific potentiator of antibiotic compositions in enhancing their activity towards Gram-negative and Gram-positive bacteria and towards fungi [262]. Structure/antibacterial activity relationships of anacardic acids have been investigated [263] by the synthesis of a series of anacardic acids with

different chain-lengths In the testing of their antimicrobial activity against *Staphylococcus aureus* the $C_{10}$ side chain compound was the most effective while in the case of *Proppionibacterium acnes*, *Streptococcus mutans* and *Brevibacterium ammoniagenes* the $C_{12}$ side chain anacardic acid was more effective. Formulated products for consumer [264-267] and non-personal use [268,269] with specific antibacterial, surfactant, and UV-absorbing properties based on salts of anacardic acid with amines and amino acids such as L-lysine, L-arginine and L-ornithine have been comprehensively examined.

Antimicrobial base materials formulated with metal and amine salts of anacardic acid, said to be non-irritative, have been proposed for cosmetic use [270]. It is of interest that anacardic acid and 2(E)-hexenal isolated from the cashew apple to which the pendant kernel (the external seed) is attached have been found to exhibit antibacterial activity against the Gram-negative bacterium, *Helicobacter pylori,* which is considered to cause acute gastritis [271]. The same compounds also inhibit the enzyme urease.

The $C_{13}$ and $C_{15}$ anacardic acids from *Pistacia vera* have been found to be antagonistic towards the fungi *Aspergillus flavus* and *Aspergillus parasiticus* [272] which can result in aflatoxins particularly in tree nut crops. Sodium salts of extracts from the cashew nutshell, rich in anacardic acids, have a potent antivectorial activity, namely the capacity to supress the transmission of disease-producing organisms between hosts [273] in the studied cases of *Aedes aegypti* larvae and *Biophalaria glabrata.* Hydrogenated compounds showed a greatly reduced activity indicating the structural importance of unsaturation.

Antialgal activity, termed zoosporicidal activity or antagonism to the asexual spores of certain algae and fungi, for example of the phytopathenogenic organism, *Aphanomyces cochliodes* by anacardic acids from unripe *Ginkgo biloba* fruit has been studied [18] and found to lead to motility inhibition and lysis of the zoospores.

*Insecticidal and Molluscicidal activity*

Compositions for the abatement of the mosquito have included anacardic acid, the primary function of which may be to enhance the shelf life through its UV absorbing properties [274]. The same might apply to formulated products with insecticidal activity, containing anacardic acid, and based on cyclodextrin inclusion complexes of neem extract [275].

Molluscicidal activity of anacardic acids, notably of the 8Z,11Z,14-triene has been reported [276] and the mechanism of its action has been studied by examining its role in initiating the action of prostacyclin ($PGI_2$) in human blood platelets through competition for receptor sites although the connectioin between the two systems may be hypothetical. More recently the molluscicidal of anacardic acids and its complexes with copper II and lead has been investigated [277].

*Enzyme inhibition by anacardic acids*

The most significant developments in biological uses of anacardic acids have been in studies of specific inhibitive action towards enzymes. Thus for example, the action of β-lactamase which cleaves the four-membered ring in penicillins, was specifically inhibited by (15:3)-anacardic acid obtained from *Spondias mombin* [45,278][68,69]. This botanical source is a little known member of the Anacardiaceae [279].

Of the (13:0), (15:1), (17:1), and (17:2)-anacardic acids, the (17:2) is the most active inhibitor [280] of glycerol 3-phosphate dehydrogenase (GPDH) and nearly comparable in action to the adipostatins [201,281][72], (15:0)-cardol derivatives, potentially of interest in human obesity studies. Inhibition of lipid biosynthesis in bacteria, yeast and animal cells by anacardic acids from *Ginkgo biloba* has also been reported by the same authors [282].

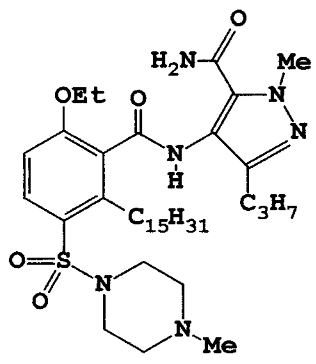
Constituents of natural cashew nutshell liquid (*Anacardium occidentale*) are active inhibitors of α-glucosidase, invertase and aldose reductase. The saturated, monoene, diene and triene anacardic acid constituents were the most potent inhibitors compared with commercially structurally-related compounds which showed a negligible activity [283].

The 8(Z) and 10(Z)-anacardic acid monoenes from *Ginkgo biloba* and also oleic, linoleic and linolenic acids have been found to be inhibitory towards soluble tissue factor (TF) VIIa complex which proteolytically activates factors IX and X leading to the generation of thrombin and fibrin clots [284]. An antithrombin-active component, namely (15:0)-anacardic acid has been isolated by bioassay-directed fractionation of the stem of *Rhus semialata* Murr variation. It exhitited activity at 50μg/ml in the amidolytic method and also prolonged the clotting time in a dose-dependent manner in the clotting assay of thrombin-fibrinogen interaction

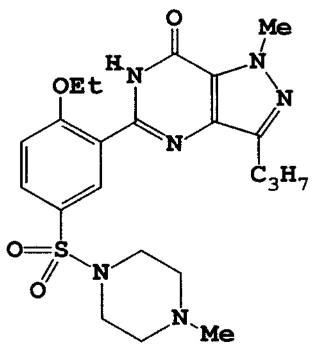
[285]. It is noteworthy that Ginkgoaceae is employed in China for protection against cardiovascular and cerebrovascular diseases.

A series of 6-oxa isosteres of anacardic acids was synthesised and several members found to be among the most potent inhibitors ($IC_{50}$ values < 5μM) of the bacterial two component regulatory systems, KinA/SpoOF and NRII/NRI reported [286].

Analogues of sildenafil (a potent phosphorodiesterase-5 ($PDE_5$) inhibitor) and an orally active drug for the treatment of erectile dysfunction] have been synthesised to find the effect of the $C_{15}$ chain on the inhibition. The activity of compound A was less than that of sildenafil, B, [287].




A




B

(17:1)-Anacardic acid of the five anacardic acids found in *Schoepfia californica* , proved to be the most effective in inhibiting the action of DNA polymerase β which can repair damage to cancer cells following clinical administration of antitumour agents, such as cis-platin, bleomycin and neocarzinostatin [41].

Regiospecific hydroperoxidation of (15:2)-anacardic acid has been effected with soy bean lipogenase to form 2-hydroxy-6-[12-hydroperoxy-(8Z,10E)-pentadecadienyl]-benzoic acid [288] but the (15:1)-monoene exhibited inhibitory action, ($IC_{50}$ ,50μM) [289]. Related work on the inhibitory properties of anacardic acids towards soybean 5-lipogenase and ovine prostaglandin endoperoxide synthase has been carried out [39]. These authors reported that 8Z-(15:1)-anacardic acid and the 10Z-(15:1)-

anacardic acid (so-called, ω5) both from the zonal geranium, *Pelargonium xhortorum* were effective inhibitors of the same two enzymes. They suggested that the natural resistance of the geranium to many arthropods and insect pests (notably aphid and spider mite) might be related to ability of the plant to synthesise anacardic acids. The possibility was discussed that anacardic acid-mediated arthropod resistance could be induced by inhibition of prostaglandin synthase and lipogenase enzymes in the insect leading to reproductive failure or physiological dysfunction. These considerations concerning anacardic acids are perhaps the most significant role for future biological and genetic developments. The function played by the anacardic acids, which are exuded by tall glandular trichromes in the geranium, *Pelargonium x hortorum* and responsible for the pest resistance , has been found to require the expression of a delta-9 14:0-acyl carrier protein fatty acid desaturase gene for their formation [44,290,291].

A series of heterocyclic derivatives of (15:0)-anacardic acid have been synthesised to investigate their activity as inhibitors of human cyclooxygenase-2 enzyme (COX-2) and towards (COX-1). Great selectivity of 5-(methoxy)-2-[(2-hydroxy-6-pentadecylphenyl)-methyl]-thio]-1H-benimidazole towards COX-2, compared with COX-1, was found [292].

*Cytotoxic action of anacardic acids*

Three anacardic acids have been isolated from the apple juice of the cashew (*Anacardium occidentale*) and examined as cytotoxic agents against BT-20 breast carcinoma cells. Anacardic acids, as well as cardols and methylcardols have been found to show moderate cytotoxic activity towards HeLa epithelioid cervix carcinoma cells and to BT-20 breast carcinoma cells [263]. To assess further the cytotoxic potential of ginkgolic acids (GA), their effect on the human keratinocyte cell line HaCaT and the rhesus monkey kidney tubular epithelial cell line LLC-$MK_2$ has been investigated *in vitro* [293]. The action of a defined mixture of GA on cell growth, viability, and integrity was evaluated by the neutral red uptake assay as well as the release of lactate dehydrogenase (LDH) and acid phosphatase (ACP). In conclusion it was recommended that for reasons of drug safety it was not considered acceptable to allow

concentrations of Ginkgo biloba to exceed technically possible levels.

*Action of Anacardic acids towards Cell components and Membranes*

Anacardic acids have been shown to have an uncoupling effect on the oxidative phosphorylation in rat liver mitochondria, the energy-responsible components of cells [294] similar to the classical uncoupler, 2,4-dinitrophenol. (15:1)-Anacardic acid was the most effective of the four acids, while salicylic acid had a very weak effect. Changes in liposomal membrane potential and pH gradient are anacardic acid-mediated and are considered to provide evidence for their having a unique function as both negative charge carriers and also 'proton carriers' that dissipate the transmembrane proton gradient formed [295]. It seems likely that these findings might be explained by the molecular changes that occur with with reduction of pH, namely, dianion →monoanion → intramolecular hydrogen-bonded structure. or at least by the two latter states.

In the marine environment there has been the first reported occurrence of so-called antifouling (namely, anticrustaceous activity) by (15:0)-anacardic acid. Thus bioassay-directed fractionation of the dichloromethane extract from twigs of *Ozoroa insignis* ,containing the phenolic lipid, was used with larvae from *Artemia salina* as a model to investigate the activity [296].

*Allergenic properties of anacardic acids and allergic contact dermatitis*

An extremely important consideration in the formulation of products for personal care is their freedom from irritative side effects and the wide range of products described, for example, in refs. [264,267-270] would appear to indicate that these materials are inocuous.

Early experimentation with *Anacardium occidentale* and cardols present in Australian species indicated that susceptibility to cardols was more prevalent than to anacardic acids or cardanols. However, more recent studies with *Ginkgo biloba* have found that anacardic acids were good sensitisers, while cardanols failed to induce allergic contact dermatitis [297].

## Biological Properties of Cardols (resorcinolic lipids)

Although resorcinolic lipids are present in an increasing number of organisms, their biological activity, physiological role and their participation in the regulation of metabolic processes do not allow the emergence of a consistent picture of their biological meaning. In view of the occurrence of these compounds in numerous products, important from the view of practical applications, there is a basic interest in determining the role of these compounds in human life as well as in the enviroment.

*Antimicrobial, antiparasitic and cytotoxic activity*

The biological activity of phenolic lipids depends significantly on the organism studied. Early in the twenties, the antibacterial action of alkyl derivatives of resorcinols (with the aliphatic chain attached at carbon 4 of the ring) was shown. They were used in treatments of infections [298-303]. Stapp also showed the bacteriostatic action of 4-hexylresorcinol on 7 different phytopathological bacterial strains [304]. Recent experiments showed that the antibacterial action of extracts from *Ginkgo biloba* fruit, *Ardisia japonica* plant, seed covers of *Myristica* fragrans or cashew nut shell liquid is related to that of resorcinolic lipids. It was shown that these compounds are highly active towards pathogenic Gram-positive bacteria, especially the acid-resistant *Mycobacterium smegmatis* [256]and *Mycobacterium tuberculosis* [152,153] as well as phytopathogenic bacteria [160,304]. A mixture of $C_{13}$ monounsaturated alkyl resorcinols and their monomethyl derivatives tested clinically on more than 200 patients showed an efficiency of above 80% in tuberculosis treatment [152]. Antibacterial activity was also shown for 5-n-alkylresorcinols and alkylresorcinolic acids isolated from the fungi *Merulius tremellous*, *Phebia radiata* [43], *Verticicladiella* sp. [198] and *Pulcherricium coeruleum* [196]. It has been demonstrated that resorcinolic lipids produced by *Pseudomonas carboxydoflava* inhibit the growth of other bacteria such as *Micrococcus lysodeictius* or *Bacillus subtilis* [305,306].

In their studies of the antibacterial activity of 5-n-pentadecylresorcinols with different degrees of the aliphatic chain unsaturation Himejima and Kubo [307] showed their characteristically strong activity towards *Streptococcus mutans*, a bacterium responsible for paradonthosis and *Propionibacterium acne*, the bacterium that causes

acne. The homologue with a saturated aliphatic chain was least active (MIC=50 μg/ml) whereas homologues with unsaturated chains showed MIC values in the range 0.78 to 1.56 μg/ml. It was also found that the presence of carboxylic groups in the ring increased the antibacterial activity remarkably and that the bactericidal activity of 4-n-hexylresorcinol towards *Streptococcus mutans* was less pronounced [308]. The activity of various resorcinolic lipids against bacteria and their use as active constituents of antiseptic lotions has been reported [309-314].

Similarly to their antibacterial activity, resorcinolic lipids exhibit fungistatic properties. However, experiments indicate that resorcinolic lipids inhibit the growth of *Trichophyton mentagrophytes* and *Saccharomyces cerevisiae* to a lower degree than observed for bacteria. No activity was shown against *Candida albicans* and *Asperigullus niger* [256]. The occurrence of 5-n-(heptadec-12-enyl) and 5-n-pentadecylresorcinols in the peel was considered responsible for the resistance of mango fruits to *Alternaria alternata* fungal infections [134,315]. Reiss in his studies on the influence of cereal grain resorcinolic lipids on growth of bread mould, showed that 5-n-pentadecylresorcinols, and likewise the 5-n-alkylresorcinol mixture from rye markedly inhibited the growth of *Aspergillus parasiticus, Aspergillus versicolor, Penicillinum chyrysogenum* and *Penicillinum roqueforte* [316]. Recently, the resistance of barley seeds to pathogenic fungi, *Aspergillus niger* and *Penicillium crysogenum*, was attributed to resorcinolic lipids present in the seed epicuticular wax. [317].

Hexylresorcinol has been used in the past as one of the best remedies for helminthiasis. Recent experiments have shown that long chain ($>C_{13}$) 5-n-alkylresorcinol homologues isolated from *Anacardium occidentale* also show molluscicidal activity against *Briomphalaria glabratus*, a parasite causing schistosomiasis, one of the serious tropical diseases [318]. The activity of alkylresorcinols was inversely proportional to the degree of unsaturation of the side chains. The most active among the studied resorcinolic lipids was 5-n-pentadecenylresorcinol (LD=7 μg/ml). The higher activity of this homologue was demonstrated against the *Filaria* class of worms. At a concentration of 3.5 μg/ml, complete eradication of parasites resulted and the alkylresorcinol was 100 times more active than diethylcarbamazine, a commonly used drug for such treatment [204].

Experimental data together with the fact that resorcinolic lipids are non-toxic to higher animals, for example, they are tolerated by rats with an oral intake of 5g/kg ([204] and Kozubek, unpublished work) has resulted in the application of these compounds, as basic components in pharmaceutical and cosmetic preparations. These preparations were found to be useful in the treatment of mouth and gingival infections, as antifungal fluids, in antiacne and also in hair restoration-lotion preparations [309,312,314].

Earlier work concerning the biological activity of 5-n-amylresorcinol (olivetol), one of the compounds formed during the thermal decomposition of marijuana, showed that this compound in concentration of 10 μM inhibits the blastogensis of human lymphocytes [319], as well as the growth of chicken embryonic heart cells in cultures [320,321]. Studies of the biological activity of phenolic lipids found in *Ginkgo biloba* (5-n-pentadecenylresorcinols) showed their strong antitumor activity against S180 tumor in mice. The active component, bilobol (Fig. 4 -**18**, 5-n-pentadec-8Z-enylresorcinol), when administered 24 hours after injection of tumour cells, in a dosage of 40 mg/kg per day for 4 days, caused almost complete inhibition of the tumor cells growth [322].

Similar activity was observed for the alkenylresorcinol against P-338 leukemia cells [97]. Systematic cytotoxic studies of biologically active compounds from the medicinal plant *Lysimachia japonica* on the KB cell cultures and tumor cells B-16, PC-13, L-5178Y, P-388, and HEp-2 showed that the resorcinolic lipids, 5-n-tridecylresorcinolic acid and 5-n-tridecylresorcinol (grevillol), were the most active components of this plant. Analysis of the $ED_{50}$, values of the effective cytotoxic concentration showed that the most active homologues were those having from 11 to 15 carbon atoms in side chains ($ED_{50}<10^{-5}$ M). It was also shown that the introduction of double bonds in the alkyl side chain of the compounds studied, increased the cytotoxic action 4 times [97]. It was also demonstrated that the presence of a carboxyl group in the resorcinol ring is not obligatory for antitumor activity as found previously for antibacterial activity [323]. These results are in agreement with earlier data concerning the role of the length and degree of unsaturation in side chains (5-n-alk(en)ylresorcinols) in inducing an increase in the permeability of biological membrane to small solutes [324].

In 1990, a new antitumor preparation comprising mainly 4-n-hexylresorcinols was elaborated [325]. The preparation administered at

the dosage of 50 μg/1g of body weight for ten days completely inhibited the growth of the tumor melanoma Cloudman S91 in mice without signs of any direct toxic side effect ($LD_{50}$>500 μg/kg). It might be expected that the replacement of hexylresorcinol by homologues with longer chains would considerably increase the therapeutic efficacy of the preparation. When the effects of 5-n-pentadec-8-enylresorcinol (bilobol) and phorbol ester (a known inducer for skin tumors) were compared on mice, it was shown that resorcinolic lipids did not induce any tumour development. Apart from some irritating effect, 5-n-pentadec-8-enylresorcinol, even at a dosage of 50 μg twice per week for 30 weeks, did not induce cancinogenic effects which were observed for phorbol ester at a dosage 20 times lower [326]. The antimicrobial (phytoanticipating) action of resorcinolic lipids has been proposed by Suzuki and Yamaguchi [327].

Recently 2-keto-alkylresorcinols from wood–inhabiting basidomycetes have been demonstrated as the active components against 8 various cancer cell lines with $IC_{50}$ values in the range from 6,9 to 63,9 μM [328]. These compounds inhibited the synthesis of both nucleic acids and proteins as demonstrated by the inhibition of the incorporation of labelled nucleotides and amino acids. Such results are in good agreement with those obtained earlier for merulinic acids by Gianetti et al. [43].

The extract of *Ginkgo biloba*, EGb 761, is known to be one of most widely used phytopharmaceutical. However, studies on the crude extract have demonstrated the presence in the preparations of ginkgolic acid, alkyl phenols and alkyl resorcinols which are, still, for some reasons, considered cytotoxic, allergenic, mutagenic and cancerogenic. In the studies concerning the possible effects of earlier compounds mentioned, Hecker et al. showed that these compounds induced inhibition of cell growth as well as the increase of the number of apoptotic cells (by 80% in relation to the control) [293]. Recently discovered in the fruits of *Ardisia colorata* derivatives, that may be considered as hydroxyl derivatives of alkylresorcinols or trihydroxybenzenes, also show cytotoxic activities, as demonstrated on FM3A cells, with the $IC_{50}$ values varied from 0.5 to 2.5 μM, depending on the structure [155]. The authors have suggested that the presence of an additional acetyl group attached to the phenolic ring might be important for their activity.

*Resorcinolic lipids as growth regulators and in host-parasite relationship*

Studies of the factors affecting the germination and growth of plants showed that the 1-ketooctyl derivative of an alkylresorcinolic acid, (Fig. 4-99), at a concentration of 3 μg/ml inhibits germination of seeds by 50%. Since this compound was isolated from a fungal plant pathogen, the authors suggested the participation of resorcinolic lipids in infections and in the killing of organisms infected by fungi [195]. The suppositions, suggesting that the occurrence of resorcinol lipid in cereal grains might be responsible for the decreased growth of animals fed with them, was raised for the first time in Wieringa's work [94]. Later results, however, showed that the main cause of a retardation in the animal growth rate is more related to an antifeedant effect, alkylresorcinol-induced decrease of the appetite [329]. The mechanism of this process, however, is not yet known.

Seeds during the period of germination and formation of shoots are susceptible to bacterial, fungal or parasite attack. The antimicrobial activity of resorcinolic lipids has been already discussed above. In some cases, a close mutual relationship has been established between host and parasite. A known example is the relationship between corn, *Sorghum*, and the parasite plant *Striga asiatica*. Infection with this parasitic plant (family *Scrophulariaceae*) causes severe damage to crop yields. The seeds of the parasite require a germination stimulus, and once germinated *Striga* survives for less than 2 weeks in the absence of a host. During studies of this problem a resorcinolic lipid derivative, xenognosin (Fig. 4-**66**) has been isolated from roots of the host plant and identified [183]. Further studies showed that its methylated congener [184] (Fig. 4-**67**) enhances the activity of the xenognosin-dependent germination signal. The importance of the role of the antioxidant properties of the compound (Fig. 4-**67**) in the infection of the host by the parasite has been discussed but the biochemical mechanism of the described process is not yet known. Additional data on the participation of alkylresorcinols in the defence system came from recent articles. Climacostol (Fig. 4-**14**) produced by *Climacostomum virens* is the lethal toxin against another predatory ciliate – *Dileptus margaritifer* [113,330]. Studies on the resistance of growing rye against pathogenic fungi indicate that alkylresorcinolic lipids may also be considered as natural fungicides. These compounds inhibited ($IC_{50}$ 8.7-9.5 μg/ml) growth of phytopathogenic fungi *Fusarium* and *Rhizoctonia* [331,332]. The activity was related to the chain lenght of the

compounds studied which, in general confirms the role of two key features of these molecules – the lenght and unsaturation of the sidechain [331]. The antimicrobial function (phytoanticipins) of resorcinolic lipids has been already proposed by Suzuki and Yamaguchi [327].

However, the fact that *Fusarium* is also able to produce and secrete alkylresorcinols [197] may suggest a more complex relationship between host and pathogen. It might be possible that some negative feedback in this relation exists – the pathogen is synthesising molecules similar to those that are lethal to it to "cheat" the host and therefore "suggesting" that the host stop or minimize the production of the proper compounds. This hypothesis, however, needs further studies.

*The effect of resorcinolic lipids on nucleic acids*

One of the possible mechanisms of the action of resorcinolic lipids on the cell, is their direct effect on the structure and metabolism of nucleic acids. Studies on heptadec-8-enylresorcinolic acid (merulinic acid A, Fig. 4-58), showed that at a concentration of 100 μg/ml it completely inhibited the synthesis of DNA, RNA, and protein synthesis in *Bacillus brevis* cells [43]. Similar inhibitory properties have been shown for 5-n-decylresorcinol which inhibited completely the synthesis of both nucleic acids in isolated rat thymocytes at a concentration of 50 μM. The protein synthesis was inhibited by 50% even at a concentration of 5 μM of this compound [333]. In studies of the anticancer properties of natural products in model systems, it was shown that resorcinolic lipids are compounds with the ability for DNA strand scission. It was demonstrated that 5-n-tridecyl and 5-n-pentadecenylresorcinols present in an extracts from the plant, *Hakea trifurcata* exhibit the ability for $Cu^{2+}$-induced scission of the replicating strand in plasmid DNA ΦX174 [147,334,335] and also of calf thymus DNA (Szmidzinski and Kozubek - unpublished data). Alkylresorcinol activity increased in the presence of oxygen, which suggested that initial oxidation of the benzene ring at $C_4$ played an important role in the described process. An increase in activity observed for homologues with longer aliphatic chains [336] indicated that the interaction of alkylresorcinol molecules with the double helix of DNA is realised through the incorporation, by intercalation of chains in its interior. The alkylresorcinol-induced nucleic acid strand scission is related to the generation of hydroxyl radicals mediated by oxidation at

high pH, in the presence of $Cu^{2+}$ and $O_2$., and alkylresorcinol [336]. The active species in DNA-scission with alkylresorcinol is believed to be the reactive oxygen species generated during the oxidation of the parent molecule. The hydroxyquinone products derived either from 1,2,4-trihydroxy-6-alkylbenzene formed during oxidation of the alkylresorcinol, or by direct one-electron oxidation of the 5-alkylresorcinol upon further reaction with oxygen and/or hydrogen peroxide would lead to the generation of the above-mentioned oxygen species active in DNA destruction. Similar structures occur in the urushiol (an alkylcatechol phenolic lipid) from *Rhus vernicifera* [337]. DNA cleavage was not a sequence-specific process and produced DNA fragments having 5'-phosphates and 3'-phosphates or 3'-phosphoroglycolates. Although this property of alkylresorcinols may be of future significance in both molecular biology and drug design, for its relevance in plant cell biology, the formation of the same products at a neutral pH and in definitely lower $Cu^{2+}$ concentration need to be demonstrated.

Recent experimental data have also shown that resorcinolic lipids, including alkylresorcinols and their derivatives at a concentration level of micrograms/ml inhibit competitively the reverse transcriptase of mice leukemia viruses and avian myeloblastosis [338,339].

Alkylresorcinols, as demonstrated by Hecht's group [334,340] are capable for mediating $Cu^{+2}$-dependent DNA cleavage and for the enhancement of the therapeutic action of antitumor drugs that function is based on the inhibition of repair of the DNA molecule. Strack et al. have shown that alkylresorcinols and bis-alkylresorcinols have the ability of potentiating the DNA strand-scission in the presence of copper ions and to enhance the effect of known antitumour activity of bleomycin [340]. Bis-alkylresorcinols, oncostemonols, (e.g., Fig. 4-**109-113**, **116-119**) have been shown as very active compound in the inhibition of growth of A2780 cells with the $IC_{50}$ values in the range of 10 μM [156]. Similar DNA-damaging effects have been shown for phenolic glycosides isolated from *Crypteronia paniculata* [341]. It may be expected that the chemical nature of phenolic lipids will support or enhance the action of DNA-damaging anticancer drugs.

*Interaction with proteins and the effects on enzymatic activity*

The ability of resorcinolic lipids to interact directly with proteins was shown in experiments with monomolecular layers of these compounds [342-344]. The monolayers prepared from alkylresorcinols mixtures with phospholipids bind proteins, especially amphiphilic ones from the subphase. The strongest binding was shown for homologues having an aliphatic chain of 19 carbon atoms in length and was considerably stronger than that observed for certain phospholipids, such as phosphatidylcholine or phosphatidylglycerol. Direct binding of alkylresorcinols to the hydrophobic regions of proteins was confirmed from the experiments, in which their ability to affect the intrinsic fluorescence intensity of tryptophan residues in such proteins as erythrocyte spectrin [345], diphtheria toxin, (Kieleczawa and Kozubek - unpublished work), the proteins of photosystems [346] and in trypsin [347] was shown. Binding of alkylresorcinols to these proteins causes a strong fluorescence quenching, indicating their localization near the tryptophan residues in the hydrophobic domains of the protein and the possibility of a direct interaction between the alkylresorcinol and the tryptophan rings.

These results and those now discussed which show the ability of resorcinolic lipids to incorporate and modulate phospholipid bilayer properties, suggest a possible similar role in biological membrane-related enzymatic activities. It has been demonstrated that at a concentration of $10^{-5}$ M, long-chain resorcinolic lipids, caused a decrease of apparent acetylocholinesterase activity in the erythrocyte membrane while simultaneously stimulating the activity of $Ca^{2+}$-dependent ATPase [348]. The inhibition of erythrocyte acetylcholinesterase has been also observed for other phenolic lipids (Stasiuk and Kozubek - unpublished work) and a similar effect of one of the homologues, namely tridecylresorcinol shown towards $Na^{+}$-$K^{+}$ ATPase [157]. $\alpha$-Glucosidase and aldolase were also inhibited by resorcinolic lipids isolated from cashew [283].

The modulating action of 5-n-alk(en)ylresorcinols upon the activity of membrane proteins may result not only from direct interaction with a protein molecule but also from alterations of their lateral mobilities. This suggestion is supported by results indicating a significant decrease of fibrinogen affinity for its receptor in platelet membranes after incubation with micromolar concentrations of various alkylresorcinol homologues

[349]. Studies of the kinetics of pancreatic phospholipase $A_2$ hydrolysis in a phosphatidylcholine bilayer modified by alkyl(en)ylresorcinol homologues also suggests the same possibility. It was shown that 5-n-heptadecyl and 5-n-heptadecenyl resorcinols when incorporated into liposomal membranes at a concentration of 4 mol%, and lower, caused a drastic increase of the latency phase of the enzyme, the period of time during which redistribution of the products within the bilayer occurs [350]. Similar inhibitory activity was observed for cobra venom phospholipase $A_2$ and the mixture of bacterial alkylresorcinols in lecithin black lipid membrane and phospholipid emulsion systems. Almost complete inhibition (95%) of the enzyme studied was observed at a concentration of about 8 mM of resorcinolic lipids [351].

Furthermore, the inhibitory effect of resorcinolic lipids ($C_{15}$-$C_{27}$) on the electron transport processes, both coupled and uncoupled in PSI and PSII photosystems also support the possibility of the action of the compounds studied in decreasing the mobility of protein molecules within the membrane. On the other hand, the occurrence of approximately 30% stimulation of coupled transport and photophosphorylation observed at a low concentration of ($0.5x10^{-7}$ M), suggests the possibility of some uncoupler properties in resorcinolic lipids [346]. The phenolic nature of resorcinolic lipids suggests the possibility of their replacement of compounds like ubiquinone or plastoquinone in mediating processes of electron and proton transport. The data on the inhibition of NADH-dependent enzymes by alkylresorcinols indicate that the molecules studied may compete with $NAD^+$ in the process of proton transport [352]. In work on compounds with antibiotic properties, derivatives of 5-n-tridecyl, 5-n-pentadecyl and 5-n-nonadecyl resorcinols sulphated on both hydroxyl groups, were isolated from fungus *Streptomyces*, and termed panosialins [96,200]. Panosialins (Fig. 4-**54**, **55**) inhibited the activity of several types of enzymes such as salidases PR8, Narashino, HVJ, acid phosphatases, and polygalactouronase. The concentrations of panosialins necessary for half-inhibition of the tested enzymes ranged from $0.6x10^{-5}$ M to $5.6x10^{-5}$ M and were dependent mainly on their aliphatic chain length [96] as was also the specificity of the inhibitory properties, particularly with respect to the type of enzyme. Thus the extent of inhibition of sialidase PR8 was inversely proportional to the chain length while the degree of inhibition of the other enzymes was directly proportional. It was also shown that $C_{15}$ 5-n-alk(en)ylresorcinols and alkylphenols from *Ginkgo biloba* exhibited inhibitory properties against

dehydrogenase enzymes like glucose-6-phosphate dehydrogenase, lactate dehydrogenase and isocitrate dehydrogenase [353]. Long chain resorcinolic lipids ($C_{10}$, $C_{19}$) at a concentration of $10^{-4}$ M also inhibited the respiration of yeast cells [344,354], *Bacillus cereus*, *Micrococcus lysodicticus* [306] and thymocytes [333] and lowered the rate of cellular oxygen uptake. Experiments on isolated mitochondria showed that long chain alkylresorcinols ($C_{19}$, $C_{25}$) also inhibited oxidation of NAD-dependent substrates [355,356], showing rotenone-like activity. Recent data indicate that 5-n-pentadecyl (Fig. 4-**5**) and 5-n-isopentadecylresorcinol (Fig. 4-**69**) from *Streptomyces* are efficient inhibitors of 3-phosphoglycerate dehydrogenase, a key enzyme of triglyceride synthesis in adipocytes [201]. Higher alkylresorcinol homologues ($C_{17}$-$C_{23}$), isolated from a cereal bran, have been found more efficient in the inhibition of this enzyme [352]. The inhibition of 3-phosphoglycerate dehydrogenase was also demonstrated for anacardic acids, the alkylphenolic acids [280,282]. Long-chain resorcinolic lipids were demonstrated to be specific inhibitors for 3-phosphoglycerate dehydrogenase (Rejman and Kozubek – submitted). These compounds were also more effective in inhibition of triglyceride accumulation in 3T3-L1 cells than other phenolic lipids studied (Rejman and Kozubek – submitted), [282].

Various cardiovascular drugs act through their effect upon $Ca^{+2}$-ATPase. The ability of resorcinolic lipid to inhibit ATPases has been show earlier [157]. Alkylphenols, alkylreosrcinols of the striatol type and other bis-alkylresorcinols (grebustols) were active in the inhibitionof $Ca^{+2}$-ATPase. The most potent inhibitors of both plasma membrane and sarcoplasmic reticulum $Ca^{+2}$-ATPases were bis-alkylresorcinols ($IC_{50}$ in the range of 10 μM). These compound were also more specific for $Ca^{+2}$-ATPase thatn for $Na^{+}$,$K^{+}$-ATPase and $Mg^{2}$-ATPase [357]. Additionally, various 5-alkylresorcinols, 5-acylresorcinols as well as 4-derivatives recently shown an ability for inhibition of one of the key enzymes of the psychiatric disorders, the monoamine oxidase (MAO) [358]. It was shown that a relatively short-chain 4-n-acylresorcinol had a $IC_{50}$ in the range of 4 μM. Despite the position of the OH-substituent and the attachment to the dihydroxybenzene ring this compound resembles olivetol, and a whole family of cannabinoid derivatives that are known for their antianxiety effects [359]. This similarity may explain a possible therapeutic effect of the single components.

*Phenolic lipids and contact dermatitis*

One of the known health problems related to naturally occurring phenolic lipids is allergic contact dermatitis which was recently discussed by Lepoittevin and Benezra [297,360,361]and 'T Hart [362]. Although most of the known problems are related mainly to catechol (urushiol type compounds) and quinone alkylphenols, similar dermatitis-inducing properties of certain alkylphenolic lipids present in Cashew Nut-Shell Liquid were reported in 1948 by Wasserman and Dawson [132]. In the last decade, Diogenes and co-workers have also described dermatitis among cashew nut workers [363,364] and Reffstrup and colleagues have reported that Philodendron-induced dermatitis is related to the presence of 5-n-heptadecenylresorcinols [36,164]. More cases of dermatitis induced by Philodendron species have been reported and the problem has been reviewed by Knight [365,366]. Additionally, a great increase in the incidence of contact dermatitis has been described due to the abundant Australian hybrid plant Grevillea "Robyn Gordon" [367] has been disclosed. However, it was demonstrated, that allergenic dermatitis related to alkylcatechols (urushiols) and alkylresorcinols might be different particularly with regard to the mechanism of induction. In clinical studies of cross-sensitivity, it appears that patients who were sensitive to Philodendron (or other alkylresorcinol-containing plants) may not necessarily be cross-sensitive to Rhus toxicodendron (and other plants containing alkylcatechols) and vice versa [365]. However, it should be pointed out that in alkylresorcinol-induced dermatitis the presence of the unsaturated chain plays a similar key role, as was similarly demonstrated for alkylcatechols [368]. Studies of the urushiol-type sensitivity showed that the formation of covalent bonds between the o-quinone from the catecholic ring and nucleophilic functionalities such as those present in proteins is responsible for enhanced presentation of the hapten to the cells of immunological system. The side chain would be responsible for binding and inserting the hapten into the cytoplasmic membrane of epidermal cells which in turn, would allow the allergen to react with membrane proteins and/or to be internalized by skin cells for activation [369,370]. Recent data of Kalergis et al. indicate that the activation of the catechol ring is related to oxidative shortening of the aliphatic side-chain, and the involvement of chain β-oxidation [371]. In considering possible

mechanisms of alkylresorcinol-induced sensitivity it should be first mentioned that since both hydroxyl groups and the chain, are in a m-position, they do not directly form o-quinones. However, during the oxidation of the dihydroxybenzene nucleus a 1,2,4-trihydroxyalkylbenzene is formed, as demonstrated by Singh et al. [336] and by work on urushiol [337]. Further oxidation of this molecule may result in formation of an o-quinonic form, active for allergy-induction. Therefore, the sensitizing activity of resorcinolic lipids appears to be, in fact, attributable to the products of their oxidation rather than to the alkylresorcinols. The diminished dermatological activity of both urushiols and alkylresorcinols with saturated chains suggests also the importance of their direct interaction with cellular membranes in manifesting biological effects.

*Interaction of resorcinolic lipids with phospholipids, bilayer and biological membranes*

Resorcinolic lipids, as well as most of other phenolic lipids have very high values of octanol/water partition coefficients [372] and can easily incorporate into the phospholipid bilayers, thereby causing considerable changes in their structure and properties. Increasing amount of resorcinolic lipids in lecithin liposomal membranes resulted in a remarkable alteration of the thermotropic properties of the phospholipids. At low membrane concentrations both saturated and unsaturated homologues showed good miscibility with phospholipids and effects related to phase separation, namely broadening of the main transition and additional phase transitions were observed with increasing concentration of alk(en)ylresorcinols in the bilayers. A shift of the main phase transition towards higher temperatures was also noted [373]. At low membrane concentrations (5-20mol%), the effect of the saturated and monounsaturated $C_{17}$ homologues, on the thermotropic properties of dipalmitoylphosphatidylcholine was different [374]. The saturated homologue, in concentration above 10 mol%, caused disappearance of the pretransition, an increase of transition temperature and enthalpy and a more than 3 fold increase in the main transition width. The unsaturated homologue caused a shift of the pretransition towards a higher temperature, similarly to the saturated homologue, with broadening of the main transition but lowering of its enthalpy. Above 15 mol% of alk(en)ylresorcinol, a process of phase separation was observed in

mixtures of phosphatidylcholine and resorcinolic lipids [373-375]. These observations have been confirmed later by Gerdon et al. [376]. Presence of incorporated resorcinolic lipids into the bilayer of lecithin vesicles also considerably affects the fluidity of the membrane from analysis of parameters involving the membrane mobility of spin-labelled fatty acids. At temperatures above the phospholipid phase transition temperature, both saturated and unsaturated homologues at concentrations lower than 6 mol% caused an increase of the order parameter value for 5-doxylstearate. At higher membrane concentrations (6-14 mol%), the homologues examined also considerably decreased 12-doxylstearate mobility [377]. An interesting observation is, that resorcinolic lipids showed a much stronger effect on the mobility of both type of markers in liposomal membranes containing cholesterol. This cholesterol-like effect of resorcinolic lipids appeared at lower membrane alk(en)yl resorcinol concentrations and was stronger for membranes containing higher cholesterol concentrations [377]. A similar stabilizing effect of alkylresorcinols was observed in diphosphatidylglycerol bilayer with pyrene as a fluorescent marker [306]. The stabilizing effect of resorcinol lipids may result from the interaction of free hydroxyl groups in the alk(en)ylresorcinol ring with phospholipids through formation of hydrogen bonds within membranes. The formation of such bonds in a mixtures of resorcinolic lipids with phospholipids was indicated by infrared spectroscopic analysis [306].

In experiments with bacterial alkylresorcinols, Batrakov and his colleagues showed that the saturated homologues could form stable black lipid membranes [351,378-380] especially at high pH (above 7.5). It was also shown that, at a pH above 8.5, 5-n-pentadec(en)ylresorcinol from cashew nut-shell liquid (cardol) forms vesicular structures, of 150 nm in diameter, that are able to entrap aqueous solutions [381,382]. Natural bacterial alkylresorcinols in the mixtures stabilized phosphatidyl-ethanolamine, phosphatidylglycerol and diphosphatidylglycerol bilayers, the extent of the stabilization being dependent on the type of phospholipid [379,380]. The authors postulated the formation in phospholipid-resorcinolic lipid mixtures of a structural network of aggregates held together by hydrogen bonding between the alkylresorcinol and the polar head groups of the phospholipids.

Alk(en)ylresorcinols, especially the unsaturated homologues, when added to media containing liposomes exerted the ability to induce an increased permeability of the bilayers towards ions and small non-

electrolytes [383]. The increased permeability of liposomal membranes induced by resorcinolic lipids may result from formation within the membrane of non-bilayer structures, such as reversed micelles or hexagonal phase ($H_{II}$ type). $^{31}$P-NMR experiments showing that unsaturated homologues at concentrations above 30mol% induced non-layer structures of the $H_{II}$ type, support this thesis [373]. It was also demonstrated with protoplasts by numerical estimation of the number of surviving hybrid cells in the minimal medium, that long-chain alkylresorcinols in the presence of calcium ions, effectively induce fusion of yeast mutant [384].

In cellular systems, resorcinolic lipids injected into the external cellular environment at concentration of $10^{-5}$ M, induce leakage of potassium ions from erythrocytes [383] and increase erythrocyte membrane permeability for non-electrolyte with a molecular diameter up to 1.4 nm [324,385]. At concentrations lower by an order of magnitude, they also induced higher membrane permeability for water [386]. Increased membrane permeability results in cell hemolysis [383,387]. By studying the relationship between the hemolytic potency of resorcinolic lipid homologs and the length and of degree of unsaturation of the aliphatic side chain, the strongest effects were shown for unsaturated $C_{15}$ and $C_{17}$ homologues [324]. Freeze-fracture electron microscopy experiments showed that changes in the distribution of proteins on the membrane surface occurred after incubation of erythrocytes with resorcinolic lipids. The $C_{15}$ saturated homologue, which induces complete release of potassium ions from erythrocyte and liposomes in test tube studies showed evident aggregation and clustering of protein particles in the membrane. Unsaturated homologues, the compounds of highest hemolytic activity, generated almost complete disruption of the membrane structure with aggregation and separation of membrane protein particles. Long chain, saturated homologues exerted the weakest effects upon membrane morphology and only minor alteration in protein distribution in the erythrocyte membrane was observed [373]. The effect of 5-n-alk(en)ylresorcinols and their derivatives on the barrier functions of biological membranes is, similarly to other amphiphilic agents, modulated by the presence of divalent cations which protect erythrocytes against the lytic action of the resorcinolic lipid [388,389]. The extent of the erythrocyte protection is dependent both on the type of cation and of the resorcinolic lipid, although $Zn^{+2}$ ions have been found to be most

active in antihemolytic protection even at $10^{-6}$M concentrations, regardless of the resorcinolic lipid type.

It should be stressed that due to very high values of buffer-membrane partition coefficients and low CMC values, the effect of resorcinolic lipids injected into the external medium is different from the effect observed when they were present internally in the membrane. For instance, the same homologues that are highly hemolytic when injected into erythrocyte suspension are not lytic when injected in the form of phosphatidylcholine-resorcinolic lipid liposomes, which indicates that direct exchange of resorcinolic lipids between membranes is limited.

Recently, Yamamoto et al. [390] reported that the biological effects of *Ginkgo biloba* extract that was encapsulated into the hybrid liposomes are enhanced. The effect of new liposomal formulations is enhanced against human lung adenocarcinoma and B-16 melanoma cells [390]. Results from our laboratory indicate that resorcinolic lipids as well as their derivatives may be an useful modifiers to the liposomal membranes with respect to the encapsulation and delivery of drugs or other bioactive components/drugs [381,382,391-396]. The liposomal pharmaceutical formulations that are connecting together amphipilic properties of resorcinolic lipids and their derivatives with the encapsulating/tissue specific properties may be considered as the new generation of supramolecular drug delivery systems.

*Phenolic lipids as modulators of lipid oxidation*

Resorcinolic lipids as phenolic compounds exhibit the ability to protect cellular lipid components from oxidation processes. It has been demonstrated that long chain 5-n-alk(en)ylresorcinol homologs prevent $Fe^{2+}$-induced peroxidation of fatty acids and phospholipids in liposomal membrane [397] as well as autooxidation processes in triglycerides and fatty acids [398,399]. Long chain resorcinolic lipid mixtures also prevent peroxidation of lipids in natural membranes. At a concentration of $10^{-3}$-$10^{-4}$ M, bacterial [305] and cereal grain [400] alkylresorcinols completely inhibited $Fe^{2+}$-ascorbic acid and $Fe^{2+}$-NADPH-induced peroxidation of liver microsomes and in fragments of the sacroplasmic reticulum [305,400]. Long-chain alkylresorcinols isolated from rye grains have also been effective in protection of the erythrocyte membrane against hydrogen peroxide-induced oxidation [342]. Alkylresorcinols present in

the lipid extracts from cereal high-fibre diet were the most active fraction showing antioxidant properties in protection of lipids against air-induced oxidation [401]. The mechanism of antioxidant action of resorcinolic lipids under physiological conditions may include formation of an intermediate 1,2,4-trihydroxy-6-alkylbenzenes [168,336] as the first product of oxidation. This compound, in turn, due to easy formation of *ortho-* and *para-* quinones may act subsequently as a more effective antioxidant [184]. Besides the phenolic ring, the length of aliphatic side-chain plays an important role in the antioxidant activity of resorcinolic lipids. The antioxidant activity of orcinol (1,3-dihydroxy-5-methylbenzene) occurs at a concentration that is at least one order of magnitude higher than that of 1,3-dihydroxy-5-pentadecylbenzene and of higher homologues isolated from cereal grains [400,402]. A recent article by Kamal-Eldin et al. [403] has confirmed previous findings by supporting some antioxidant properties of cereal alkylresorcinols. On the other hand, this paper showed that the antioxidant properties of the native alkylresorcinols are weak, which is in good agreement with previous data, e.g. [397,398]. Nevertheless, the suggested consumption of the high-fibre diet products by humans and resulting intake of alkylresorcinols and their derivatives should be taken into account is spite of the possible biological consequences.

The inhibitory action of alkylresorcinols on phospholipase $A_2$ suggests also the possibility of the participation of these compounds in the modulation of enzymatic oxidation of lipids leading to formation of metabolically active products as for example leukotrienes, thromboxanes and prostaglandins. Experiments on the effect of saturated and unsaturated 5-n-alkylresorcinol homologues upon the oxidation of arachidonic acid by leukocyte lipoxygenase (5-LOX) and cyclooxygenase from seminal vesicles, showed that at a concentration of 50μM, lipoxygenase is effectively inhibited (90%) by polyunsaturated pentadecylresorcinol homologues [39,404,405]. The inhibitory activity of grevillol (5-n-tridecylresorcinol) (Fig. 4-4) was twice lower than that of the $C_{15}$ homologue. Cyclooxygenase did not show such inhibitory dependence on the degree of unsaturation of the resorcinolic lipid aliphatic chains and the inhibitory activity of mono, di, and tri-unsaturated homologs was similar. Cyclooxygenase was strongly inhibited at low concentrations of resorcinolic compounds and pentadeca-8,11,14-trienylresorcinol at a concentration of 10 μM inhibited

this enzyme almost completely [404]. By contrast with other activities, methyl derivatives of pentadecylresorcinol showed inhibitory properties upon cyclooxygenase activity (87% inhibition at 10μM). Long-chain 5-n-alkylresorcinol homologues showed also high inhibitory potencies against soybean lipoxygenase isoenzymes [406,407] which was dependent both on the chain length and the degree of side-chain unsaturation as well as on the isoenzyme studied and the substrate used. It has been shown that resorcinolic lipids isolated from cereals can modulate the synthesis of thromboxane A in platelets [408]. The complex dependence of the amount of synthesized thromboxane on alkylresorcinol concentration which indicated that high and low concentrations stimulated whereas an average concentrations inhibited, suggest the need for more detailed studies of the relationship between the level of peroxides in the cells, alkylresorcinols concentration and thromboxane synthesis. The inhibitory properties of resorcinolic lipids upon lipoxygenases also indicate the possible participation of these compounds in plant apoptotic/senescence events as well as the possible application of these natural compounds in treatment of diseases in which lipoxygenases play a major role.

Mutagenesis induced by xenobiotics in many cases is related to their metabolic activation via oxidation processes. The lack of a cancinogenic effect of alkylresorcinols [326,409] together with their antioxidant properties suggests their possible participation in the protection of cells against carcinogenesis. A preparation, comprising a mixture of predominantly saturated-chain homologues ($C_{15}$-$C_{27}$, with average chain length of 18.4 carbon atoms), drastically inhibited the effect of direct and indirect (metabolically activated) mutagens. The effect was strongest in the case of the indirect-acting mutagens, benzo(α)pyrene and 2-aminofluorene where, in the Ames test, already at a doses of 10 μg/plate, more than 50% inhibition was observed. For direct-acting mutagens, such as methyl methanesulfonate and, especially, daunorubicin, the effect of resorcinolic lipids was smaller but still noticeable. In the Sister Chromatid Exchanges test (SCEs) with cultured *in vitro* human blood-derived lymphocytes, a significant decrease of the SCEs frequency induced by benzo(α)pyrene, was also observed [410,411]. Recent data of George and Kuttan on cashew nut shell liquid phenolic lipids confirmed the lack of mutagenic, carcinogenic and cocarcinogenic activities of these compounds [409].

It should be mentioned that in many cases direct comparisons of reported biological effects are very difficult. Due to amphiphilic properties, a significant part of the effect of resorcinolic lipids is related to their interaction with membranous structures and the hydrophobic domains of proteins and therefore, the molar ratios rather than absolute concentrations should be used as a actual measure. The importance of this fact is clearly observed, e.g., in the studies of hemolytic concentration dependence on the number of erythrocytes used in the tests and in the time dependence of this process [387].

Recently discovered in the fruits of *Ardisia colorata,* derivatives (ardisiphenols) that may be considered as hydroxyl derivatives of alkylresorcinlos or trihydroxybenzene drivatives, these compound, beside their cytotoxic activities, show antioxidant properties. Although these properties are not strong (the Trolox equivalent does not exceed the value of 0.5) their biologial acivity as cytotoxic compounds showed them to be important ($IC_{50}$ values from 0,5 to 2,8 μM) [155]

*Resorcinolic lipids as biomarkers of whole grain wheat and rye intake*

Recent studies by Ross et.al. have demonstrated that resorcinolic lipids (5-alkylresorcinols) present in high fibre whole grain cereal diet can be used as biomarkers, i.e. compounds that can be used for the epidemiological studies of the effects of dietary intake in human health [412-415]. These phenolic compounds are absorbed from the ileum by animals and humans and may be determined quantitatively in the serum [416] and their metabolites also in human urine [412]. Further data confirm that cereal grain 5-alkylreesorcinols modify animal lipid metabolism *in vivo* and *in vitro* [413,417] which make them very interesting in relation to the risks of diabetes, obesity, heart diseases and some cancers.

## CONCLUSIONS

A wide range of botanical and biological families are sources of phenolic lipids. Notable classes of compounds present are certain dihydric phenols (the cardols), phenolic acids (the anacardic acids) and a variety of

monohydric phenols. Many of their chemical structures are common to a large proportion of these natural species and perhaps suggest the basis of a chemical taxonomy. Some families are prominent, notably *Anacardium occidentale* as raw materials in both technical practice and in studies on bioactivity which have achieved significant results. As with all natural organic compounds, beneficial physiological activity has always to take regard of toxicological considerations. In the present surge of activity in the study of the bioactivity of phenolic lipids there does not appear to be compelling evidence of adverse findings to deter this progress.

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## REFERENCES

[1] Tyman, J. H. P. In *Studies in Natural Products Chemistry*; Atta-Ur-Rahman, Ed.; Elsevier: Amsterdam, **1991**; Vol. 9.
[2] Tyman, J. H. P. *Synthetic and Natural Phenols*; Elsevier: Amsterdam, **1996**.
[3] Kozubek, A.; Tyman, J. H. P.; *Chemical Reviews* **1999**, *99*, 1 - 26.
[4] Tyman, J. H. P.; *Recent Res. Devel. Lipids* **2001**, *5*, 125-145.
[5] Tyman, J. H. P.; *Recent Res. Devel. Lipids* **2001**, *5*, 125-145.
[6] Furukawa, S.; *Sci. Papers Inst. Phys. Chem. Res. (Tokyo)* **1934**, *24*, 304 - 327.
[7] Han, D.-S.; *Soul Uidae Chapchi* **1964**, *5*, 281 -290.
[8] Kawamura, J.; *Japan J. Chem.* **1928**, *3*, 89 - 95.
[9] Amico, V.; Biondi, D.; *J. Nat. Prod.* **1990**, *53*, 1379 - 1382.
[10] Anderson, H. H.; David, N. A.; Leake, C. D.; *Proc.Soc.Exp.Biol.Med.* **1931**, *28*, 609 - 612.
[11] Tyman, J. H. P.; *Chem. Soc. Rev.* **1979**, *8*, 499-537.
[12] Backer, H. J.; Haack, N. H.; *Rec. Trav. Chim. Pays-Bas* **1941**, *60*, 661 - 677.
[13] Harvey, M. T.; Caplan, S.; *Indian. J. Chem.* **1940**, *32*, 1306-1308.
[14] Symes, W. F.; Dawson, C. R.; *Nature* **1953**, *171*, 841 - 842.
[15] Lamberton, J. A.; *Austral. J. Chem.* **1958**, *11*, 73-84.
[16] Lamberton, J. A.; *Austral. J. Chem.* **1959**, *12*, 234-236.
[17] Loev, B.; Dawson, C. R.; *J. Am. Chem. Soc.* **1958**, *80*, 643 - 645.
[18] Begum, P.; Hashidoko, Y.; Tofazzal Islam, M.; Ogawa, Y.; Tahara, S.; *Z. Naturforsch. C* **2002**, *57c*, 874-882.
[19] Pham, V. C.; Jossang, A.; Sevenet, T.; Bodo, B.; *Tetrahedron* **2002**, *58*, 5709-5714.
[20] Pham, V. C.; Jossang, A.; Sevenet, T.; Bodo, B.; *Tetrahedron* **2000**, *56*, 1707-1713.

[21] Wilson, D. M.; Fenical, W.; Hay, M.; Lindquist, N.; Bolser, R.; *Phytochemistry* **1999**, *50*, 1333-1336.
[22] Achenbach, H.; Witzke, J.; *Angew. Chem. Internat. Edn.* **1977**, *89*, 198-201.
[23] Hartmann, S.; Minnikin, D. In *Surfactants in Lipid Chemistry*; Tyman, J. H. P., Ed.; Royal Society of Chemistry: Cambridge, **1992**.
[24] Bu'lock, J. D.; Hudson, A. T.; *J. Chem. Soc.* **1969**, *60*, 61-63.
[25] Juagdan, E. G.; Kalidindi, R. S.; Scheuer, P. J.; *Tetrahedron Lett.* **1995**, *36*, 2905-2908.
[26] Kushlan, D. M.; Faulkner, D. J.; *J. Nat. Prod.* **1991**, *54*, 1451-1454.
[27] Sleeper, H. L.; Fenical, W.; *J. Am. Chem. Soc.* **1977**, *99*, 2367-2368.
[28] Lam, S. K.; Tyman, J. H. P.; *Lipids* **1978**, *13*, 525-.
[29] Paul, V. J.; Yeddanapalli, L. M.; *Nature* **1954**, *174*, 604.
[30] Tyman, J. H. P.; Jacobs, N.; *J. Chromatogr.* **1971**, *54*, 83 - 90.
[31] Sharma, N. K.; Sharma, V. N.; *Indian J. Chem.* **1966**, *4*, 504-506.
[32] Kubo, I.; Kim, M.; Naya, K.; Komatsu, S.; Yamagiwa, Y.; Ohashi, K.; Sakamoto, Y.; Hirakawa, S.; Kamikawa, T.; *Chem. Lett.* **1987**, 1101 -1104.
[33] Yalpani, M.; Tyman, J. H. P.; *Phytochemistry* **1983**, *22*, 2263 - 2266.
[34] Corthout, J.; Pieters, L.; Claeys, M.; Geerts, S.; Vandenberghe, D.; Vlietinck, A.; *Planta Medica* **1994**, *60*, 460-463.
[35] Spencer, G. F.; Tjarks, L. W.; Kleiman, R.; *J. Nat. Prod.* **1980**, *43*, 724 - 730.
[36] Reffstrup, T.; Hammershoy, O.; Boll, P. M.; Schmidt, H.; *Acta Chem. Scand.* **1982**, *B 36*, 291 - 294.
[37] Bohlmann, F.; Kleine, K. M.; *Ber.* **1962**, *95*, 602-608.
[38] Grazzini, R. A.; Paul, P. R.; Hage, T.; Cox-Foster, D. L.; Medford, J. I.; Craig, R.; Mumma, R. O.; *J. Chem. Ecol.* **1999**, *25*, 955-963.
[39] Grazzini, R.; Hesk, D.; Heininger, E.; Hildebrandt, G.; Reddy, C. C.; Cox-Foster, D.; Medford, J.; Craig, R.; Mumma, R. O.; *Biochem. Biophys. Res. Commun.* **1991**, *176*, 775 - 780.
[40] Grazzini, R.; Hesk, D.; Yerger, E.; Cox-Foste, D.; Medford, J.; Craig, R.; Mumma, R. O.; *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 343-346.
[41] Chen, J.; Zhang, Y.-H.; Wang, L.-K.; Sucheck, S. K.; Snow, A. M.; Hecht, S. M.; *Chem. Commun.* **1998**, 2769-2770.
[42] Asahina, Y.; Miyasaka, M.; Sekisawa, T.; *Ber.* **1936**, *69B*, 1643-1646.
[43] Giannetti, B. M.; Steglich, W.; Quack, W.; Anke, T.; Oberwinkler, F.; *Z. Naturforsch.* **1978**, *33c*, 807 - 816.
[44] Schultz, D. J.; Cahoon, E. B.; Shanklin, J.; Craig, R.; Coxfoster, D. L.; Mumma, R. O.; Medford, J. I.; *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8771-8775.
[45] Coates, N. J.; Gilpin, M. L.; Gwynn, M. N.; Lewis, D. E.; Milner, P. H.; Spear, S. R.; Tyler, J. W.; *J. Nat. Prod.* **1994**, *57*, 654-657.
[46] Asahina, T.; Fuzikawa, F.; *Ber.* **1935**, *68B*, 80-85.
[47] Adawadkar, P. D.; Elsohly, M. A.; *Phytochemistry* **1983**, *22*, 1280 - 1281.
[48] Anaya, A. L.; Mata, R.; Rivero-Cruz, F.; Hernandez-Bautista, B. E.; Chavez-Velasco, D.; Gomez-Pompa, A.; *Journal of Chemical Ecology* **1999**, *25*, 141-156.

[49] Ale, S.; Ferreira, F.; Gonzalez, G.; Epstein, W.; *Am J Contact Dermat* **1997**, *8*, 144-9.
[50] Baer, H.; Hooton, M.; Fales, H.; Wu, A.; Schaub, F.; *Phytochemistry* **1980**, *19*, 799 - 802.
[51] Billets, S.; Craig, J. C.; Corbett, M. D.; Vickery, J. F.; *Phytochemistry* **1976**, *15*, 533 - 535.
[52] Byck, J.; Dawson, C.; *J Org Chem* **1967**, *32*, 1084-8.
[53] Byers, V.; Epstein, W.; Castagnoli, N.; Baer, H.; *J Clin Invest* **1979**, *64*, 1437-48.
[54] Byers, V. S.; Castagnoli, N. J.; Epstein, W. L.; *J. Clin. Invest.* **1979**, *64*, 1449 - 1456.
[55] Corbett, M.; Billets, S.; *J Pharm Sci* **1975**, *64*, 1715-8.
[56] Craig, J.; Waller, C.; Billets, S.; Elsohly, M.; *J Pharm Sci* **1978**, *67*, 483-5.
[57] Du, Y.; Oshima, R.; Kumanotani, J.; *J. Chromatogr.* **1984**, *284*, 463 - 467.
[58] Dunham, P.; Vosshall, L.; Bayer, C.; Rich, A.; Weissmann, G.; *Fed Proc* **1985**, *44*, 2914-24.
[59] Dunn, I.; Liberato, D.; Castagnoli, N.; Byers, V.; *Cell Immunol* **1982**, *74*, 220-33.
[60] Dunn, I.; Liberato, D.; Dennick, R.; Castagnoli, N.; Byers, V.; *Cell Immunol* **1982**, *68*, 377-88.
[61] Dunn, I. S.; Liberato, D. J.; Castagnoli, N.; Byers, V. S.; *Cell. Immunol.* **1982**, *74*, 220 - 233.
[62] Dupuis, G.; *Br J Dermatol* **1979**, *101*, 617-24.
[63] Elsohly, M.; Turner, C.; *J Pharm Sci* **1980**, *69*, 587-9.
[64] Elsohly, M. A.; Adawadkar, P. D.; Ma, C.-Y.; Turner, C. E.; *J. Nat. Prod.* **1982**, *45*, 532 - 538.
[65] Elsohly, M.; Adawadkar, P.; Benigni, D.; Watson, E.; Little Tl, J.; *J Med Chem* **1986**, *29*, 606-11.
[66] Gross, M.; Baer, H.; Fales, H. M.; *Phytochemistry* **1975**, *14*, 2263 - 2266.
[67] Halim, H.; Locksley, H. D.; Memon, J. J.; *J. Chem. Soc. Perkin Trans. 1.* **1980**, 2331 - 2337.
[68] Jaggy, H.; Koch, E.; *Pharmazie* **1997**, *52*, 735-738.
[69] Jefferson, A.; Wangchareontrakul, S.; *J. Chromatogr.* **1986**, *367*, 145 - 154.
[70] Jefferson, A.; Wangchareontrakul, S.; *J. Chromatogr.* **1986**, *367*, 145 -154.
[71] Jinno, S.; Okita, T.; *Chemical & Pharmaceutical Bulletin* **1998**, *46*, 1688-1694.
[72] Johnson, R.; Haer, H.; Kirkpatrick, C.; Dawson, C.; Khurana, R.; *J Allergy Clin Immunol* **1972**, *49*, 27-35.
[73] Kalish, R.; Morimoto, C.; *J Clin Invest* **1988**, *82*, 825-32.
[74] Kawai, K.; Nakagawa, M.; Kawai, K.; Miyakoshi, T.; Miyashita, K.; Asami, T.; *Contact Dermatitis* **1992**, *27*, 244-9.
[75] Lin, Q.; Xu, K.; Hu, B.; Peng, H.; Liu, X.; *Kexue Tongbao (Foreign Lang. Ed.)* **1984**, *29*, 485 - 489.
[76] Marks Jg, J.; Demelfi, T.; Mccarthy, M.; Witte, E.; Castagnoli, N.; Epstein, W.; Aber, R.; *J Am Acad Dermatol* **1984**, *10*, 627-31.
[77] Marks Jg, J.; Trautlein, J.; Epstein, W.; Laws, D.; Sicard, G.; *Arch Dermatol* **1987**, *123*, 476-8.

[78] Symes, W. F.; Dawson, C. R.; *J. Org. Chem.* **1954**, *76*, 2959 - 2963.
[79] Watson, E.; Murphy, J.; Wirth, P.; Waller, C.; Elsohly, M.; *J Invest Dermatol* **1981**, *76*, 164-70.
[80] Weissmann, G.; Azaroff, L.; Davidson, S.; Dunham, P.; *Proc Natl Acad Sci U S A* **1986**, *83*, 2914-8.
[81] Majima, R.; *Ber.* **1915**, *48*, 1593-1597.
[82] Majima, R.; *Ber.* **1922**, *55B*, 191-214.
[83] Markiewitz, K. H.; Dawson, C. R.; *J. Org. Chem.* **1965**, *30*, 1610-1613.
[84] Majima, R.; Cho, S.; *Ber.* **1907**, *40*, 4390-4393.
[85] Backer, H. J.; Haack, N. H.; *Rec. Trav. Chim. Pays-Bas* **1938**, *57*, 225 - 232.
[86] Du, Y.; Oshima, R.; *J. Chromatogr.* **1985**, *318*, 378 - 383.
[87] Du, Y.; Oshima, R.; Yamauchi, Y.; Kumanotani, J.; Miyakoshi, T.; *Phytochemistry* **1986**, *25*, 2211 - 2218.
[88] Majima, R.; Nakamura, I.; *Ber.* **1915**, *48*, 1597-1603.
[89] Backer, H. J.; Haack, N. H.; *Rec. Trav. Chim. Pays-Bas* **1941**, *60*, 656 - 660.
[90] Gorst-Allman, C. P.; Steyn, P. S.; Heyl, T.; Wellsand, M. J.; Fourie, D. M. C.; *S. Afr. J. Chem.* **1987**, *40*, 82-89.
[91] Shoji, N.; Iwasa, A.; Takemoto, T.; Ishida, Y.; Ohizumi, Y.; *J. Pharm Sci.* **1982**, *71*, 174.
[92] Silberborth, S.; Stumpf, A.; Erkel, G.; Anke, T.; Sterner, O.; *Phytochemistry* **2002**, *59*, 643-648.
[93] Wenkert, E.; Loeser, E.-M.; Mahapatra, S. N.; Schenker, F.; Wilson, E. M.; *J. Org. Chem.* **1964**, *29*, 435 - 439.
[94] Wieringa, G. W. *On the occurrence of growth inhibiting stubstances in rye.*; Veenman H.: Wageningen, **1967**.
[95] Briggs, D. E.; *Phytochemistry* **1974**, *13*, 987 - 996.
[96] Aoyagi, T.; Yagisawa, M.; Kumagai, M.; Hamada, M.; Okami, Y.; Takeuchi, T.; Umezawa, H.; *J. Antibiot.* **1971**, *24*, 855 - 860.
[97] Arisawa, M.; Ohmura, K.; Kobayashi, A.; Morita, N.; *Chem. Pharm. Bull.* **1989**, *37*, 2431 - 2431.
[98] Asahina, Y.; M., Y.; *Ber.* **1937**, *70*, 206-209.
[99] Evans, L. E.; Dedio, W.; Hill, R. D.; *Can. J. Plant Sci.* **1973**, *53*, 485 - 488.
[100] Gohil, S.; Pettersson, D.; Salomonsson, A.-C.; Aman, P.; *J. Sci. Foof. Agric.* **1988**, *45*, 43 - 52.
[101] Hengtrakul, P.; Lorenz, K.; Mathias, M.; *J. Food Comp. Anal.* **1991**, *4*, 52 - 57.
[102] Musehold, J.; *Z. Pflanzenzuchtg.* **1978**, *80*, 326 - 332.
[103] Musehold, J.; *Z. Pflanzenzuchtg.* **1974**, *71*, 124 - 129.
[104] Batrakov, S. G.; Pridachina, N. N.; Kryglyak, E. B.; Novogrudskaya, E. D.; *Khim. Prirod. Soed. (USSR)* **1977**, *4*, 494-499.
[105] Reusch, R. N.; Sadoff, H. L.; *J. Bacteriol.* **1979**, *139*, 448 - 453.
[106] Reusch, R. N.; Sadoff, H. L.; *Nature* **1983**, *302*, 268 - 270.
[107] Kozubek, A.; Pietr, S.; Czerwonka, A.; *J. Bacteriol.* **1996**, *178*, 4027-4030.
[108] Kanda, N.; Ishizaki, N.; Inoue, N.; Oshima, M.; Handa, A.; Kitahara, T.; *J. Antibiot.* **1975**, *28*, 935 - 942.
[109] Kitahara, T.; Kanda, N.; *J. Antibiot.* **1975**, *28*, 943 - 946.

[110] Osipov, G. A.; El-Registan, G. I.; Svetlichny, V. A.; Kozlova, A. N.; Duda, V. I.; Kaprelyants, A. S.; Pomazanov, V. V.; *Mikrobiologiya (Russ)* **1985**, *54*, 186-190.
[111] Budzikiewicz, H.; Scholl, H.; Neuenhaus, W.; Pulverer, G.; Korth, H.; *Z. Naturforsch.* **1980**, *35b*, 909-910.
[112] Barrow, R. A.; Capon, R. J.; *Aust. J. Chem.* **1991**, *44*, 1393-1405.
[113] Masaki, M. E.; Harumoto, T.; Terazina, M. N.; Miyake, A.; Usuki, Y.; Iio, H.; *Tetrahedron Lett.* **1999**, *40*, 8227-8229.
[114] Tyman, J. H. P.; Visani, N.; *Chem. Phys. Lipids* **1997**, *85*, 157-174.
[115] Hengtrakul, P.; Lorenz, K.; Mathias, M.; *Cereal Chem.* **1990**, *67*, 413 - 417.
[116] Lorenz, K.; *Proc. 10th Natl. Conf. Wheat Util. Res.* **1977**, *U.S. Dept. Agr., Agr. Rev. Manuals*, 72 - 80.
[117] Lorenz, K.; Al Ruqaie, I.; *Food Sci. Technol.* **1992**, *25*, 248-252.
[118] Nowacki, E.; Aniol, A.; *Hodowla Rosl., Aklim. Nasienn.* **1975**, *19*, 609 - 616.
[119] Musehold, J.; *Hodowla Rosl. Aklim. Nasienn.* **1975**, *19*, 603 - 604.
[120] Verdeal, K.; Lorenz, K.; *Cereal Chem.* **1977**, *54*, 475 - 483.
[121] Tyman, J. H. P.; *Anal. Chem.* **1976**, *48*, 30 - 34.
[122] Mullin, W. J.; Emery, J. P. H.; *J. Agr. Food Chem.* **1992**, *40*, 2127-2130.
[123] Kozubek, A.; *J. Chromatogr.* **1984**, *295*, 304 - 307.
[124] Kozubek, A.; Tluscik, F.; Mejbaum-Katzenellenbogen, W.; *Acta Soc. Bot. Polon.* **1981**, *50*, 637 - 643.
[125] Kozubek, A.; Geurts Van Kessel, W. S. M.; Demel, R. A.; *J. Chromatogr.* **1979**, *169*, 422 - 425.
[126] Kozubek, A.; *Acta Aliment. Polon.* **1985**, *9*, 185 - 198.
[127] Mullin, W. J.; Collins, F. W.; *J. Food Comp. Anal.* **1991**, *4*, 270-275.
[128] Batrakov, S. G.; Pridachina, N. N.; Kruglyak, E. B.; Novogrudskaya, E. D.; Chekasina, E. V.; *Izv. Akad. Nauk SSSR Ser. Biol.* **1984**, 177 - 184.
[129] Reusch, R. N.; Sadoff, H. L.; *J. Bacteriol.* **1981**, *145*, 889 - 895.
[130] Suzuki, Y.; Esumi, Y.; Uramoto, M.; Kono, Y.; Sakurai, A.; *Biosci. Biotech. Biochem.* **1997**, *61*, 480-486.
[131] Tyman, J. H. P.; *J. Chem. Soc., Perkin I* **1973**, 1639 - 1647.
[132] Wasserman, D.; Dawson, R.; *J. Am. Chem. Soc.* **1948**, *70*, 3675 - 3679.
[133] Bandyopadhyay, C.; Gholap, A.; Mamdapur, V. R.; *J. Agric. Food Chem.* **1985**, *33*, 377 - 379.
[134] Cojocaru, M.; Droby, S.; Glotter, E.; Goldman, A.; Gottlieb, H. E.; Jacoby, B.; Prusky, D.; *Phytochemistry* **1986**, *25*, 1093 - 1095.
[135] Mamdapur, V. R.; *J. Agric. Food Chem.* **1985**, *33*, 377 - 379.
[136] Morimoto, H.; Kawamatsu, Y.; Sugihara, H.; *Chem. Pharm. Bull.* **1968**, *16*, 2282-2286.
[137] Gellerman, J. L.; Anderson, W. H.; Schlenk, H.; *Phytochemistry* **1976**, *15*, 1959 - 1961.
[138] Cirrigotis, K. A.; Cleaver, L.; Corrie, J. E. T.; Grasby, R. G.; Green, G. H.; Mock, J.; Nimgirawath, S.; Read, R. W.; Ritchie, E.; Taylor, W. C.; Vadasz, A.; Webb, W. R. G.; *Aust. J. Chem.* **1974**, *27*, 345 - 355.
[139] Occolowitz, J. L.; *Analyt. Chem.* **1964**, *36*, 2177.
[140] Occolowitz, J. L.; Wright, A. S.; *Aust. J. Chem.* **1962**, *15*, 858 - 861.

[141] Ridley, D. D.; Ritchie, E.; Taylor, W. C.; *Aust. J. Chem.* **1968**, *21*, 2979 - 2988.
[142] Ritchie, E.; Taylor, W. C.; Vautin, S. T. K.; *Aust. J. Chem.* **1965**, *18*, 2015 - 2020.
[143] Varma, R. S.; Manju, M.; Parthasarathy, M. R.; *Phytochemistry* **1976**, *15*, 1418 - 1419.
[144] Ahmed, A. S.; Nakamura, N.; Meselhy, M. R.; Makhboul, M. A.; El-Emary, N.; Hattori, M.; *Phytochemistry* **2000**, *53*, 149.
[145] Barr, J. R.; Scannell, R. T.; Yamaguchi, K.; *J. Org. Chem.* **1989**, *54*, 494 - 496.
[146] Manju, M.; Parthasarathy, M. R.; *Indian J. Chem. Sect. B* **1977**, *15*, 1090 - 1093.
[147] Scannell, R. T.; Barr, J. R.; Murty, V. S.; Reddy, K. S.; Hecht, S. M.; *J. Am. Chem. Soc.* **1988**, *110*, 3650 - 3651.
[148] Bullock, L. M.; Drewes, S. E.; *S. Afr. J. Chem.* **1989**, *42*, 76 - 80.
[149] Cannon, J. R.; Metcalf, B. W.; *Austral. J. Chem.* **1971**, *24*, 1925 - 1927.
[150] Madrigal, R. V.; Spencer, G. F.; Plattner, R. D.; Smith, C. R. J.; *Lipids* **1977**, *12*, 402 - 406.
[151] Huang, P.-H.; Chen, W.-S.; Hu, Y.; *Yao Hsueh T'ung Pao* **1980**, *15*, 39.
[152] Hu, Y.; Chen, W.-S.; Huang, B.-H.; Lin, L.-Z.; Xu, R.-S.; *Hua Hsueh Hsueh Pao* **1981**, *39*, 153 - 158.
[153] Hu, Y.; Chen, W.-S.; Huang, B.-H.; Lin, L.-Z.; Xu, R.-S.; *K'o Hsueh T'ung Pao* **1979**, *24*, 907 - 909.
[154] Sumino, M.; Sekine, T.; Ruangrungsi, N.; Igarashi, K.; Ikegami, F.; *Chem. Pharm. Bull.* **2002**, *50*, 1484-1487.
[155] Sumino, M.; Sekine, T.; Ruangrungsi, N.; Ikegami, F.; *Chem. Pharm. Bull.* **2002**, *49*, 1664-1665.
[156] Chaturvedula, V. S. P.; Schilling, J. K.; Miller, J. S.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I.; *J. Nat. Prod.* **2002**, *65*, 1627-1632.
[157] Shoji, N.; Umeyama, A.; Takemoto, T.; *J. Nat. Prod.* **1984**, *47*, 530 - 532.
[158] Gonzalez, M. J. T. G.; Deoliveira, C. J. C.; Fernandes, J. O.; Kijjoa, A.; Herz, W.; *Phytochemistry* **1996**, *43*, 1333-1337.
[159] Kato, M. J.; Lopes, L. M. X.; Fo, H. F. P.; Yoshida, M.; Gottlieb, O. R.; *Phytochemistry* **1985**, *24*, 533-536.
[160] Orabi, K. Y.; Mossa, J. S.; El-Feraly, F. S.; *J. Nat. Prod.* **1991**, *64*, 856 - 859.
[161] Marini-Bettolo, G. B.; Delle Monache, F.; Goncalves Da Lima, O.; De Barros Coelho, S.; *Gazz. Chim. Ital.* **1971**, *101*, 41.
[162] Kato, T.; Seki, K.; Kaneko, R.; *Colloid Polym. Sci.* **1990**, *268*, 934 - 937.
[163] Dring, J. V.; Kite, G. C.; Nash, R. J.; Reynolds, T.; *Bot. J. Linn. Soc.* **1995**, *117*, 1-12.
[164] Reffstrup, T.; Boll, R. M.; *Phytochemistry* **1985**, *24*, 2563 - 2565.
[165] Bohlmann, F.; Wegner, P.; *Phytochemistry* **1982**, *21*, 1693 - 1695.
[166] Brown, G. D.; *J. Nat. Prod.* **1992**, *55*, 1756-1760.
[167] Bohlmann, F.; Kramp, W.; Grenz, M.; Robinson, H.; King, R. M.; *Phytochemistry* **1981**, *20*, 1907 - 1913.
[168] Dupre, S.; Bohlmann, F.; Knox, E.; *Biochem. Syst. Ecol.* **1990**, *18*, 149 - 150.
[169] Barrero, A.; Sanchez, J. F.; Barron, A.; Corrales, F.; Rodriguez, I.; *Phytochemistry* **1989**, *28*, 161 - 164.

[170] Barrero, A. F.; Cabrera, E.; Rodriguez, I.; Fernandezgallego, E. M.; *Phytochemistry* **1994**, *36*, 189-194.

[171] Barrero, A. F.; Cabrera, E.; Rodriguez, I.; Planelles, F.; *Phytochemistry* **1994**, *35*, 493-498.

[172] Barrero, A. F.; Herrador, M. M.; Arteaga, P.; Rodriguez-Garcia, I.; Garcia-Moreno, M.; *J. Nat. Prod.* **1997**, *60*, 65-68.

[173] Barrero, A. F.; Sanchez, J. F.; Reyes, F.; Rodriguez, I.; *Phytochemistry* **1991**, *30*, 641 - 643.

[174] Barrero, A. F.; Sanchez, J. F.; Rodriguez, I.; *Phytochemistry* **1990**, *29*, 1967 - 1969.

[175] Barrero, A. F.; Sanchez, J. F.; Rodriguez, I.; Maqueda, M.; *J. Nat. Prod.* **1993**, *56*, 1737-1746.

[176] Zarnowski, R.; Kozubek, A.; *Z. Naturforsch. C* **1999**, *54*, 44-48.

[177] Seitz, L. M.; Love, G. R.; *Cereal Foods World* **1987**, *32*, 650.

[178] Seitz, L. M.; *J. Agric. Food Chem.* **1992**, *40*, 1541-1546.

[179] Tluscik, F.; Kozubek, A.; *Chem. Anal. (Warsaw)* **1984**, *29*, 79 - 84.

[180] Kaczmarek, J.; Tluscik, F.; *Genet. Polon.* **1984**, *25*, 349 - 358.

[181] Kozubek, A.; Tyman, J. H. P.; *Chem. Phys. Lipids* **1995**, *78*, 29-35.

[182] Zarnowski, R.; Suzuki, Y.; Yamaguchi, I.; Pietr, S. J.; *Z. Naturforsch. C* **2002**, *57c*, 57-62.

[183] Chang, M.; Netzly, D. H.; Butler, L. G.; Lynn, D. G.; *J. Am. Chem. Soc.* **1986**, *108*, 7858-7860.

[184] Fate, G. D.; Lynn, D. G.; *J.Am. Chem. Soc.* **1996**, *118*, 11369-11376.

[185] Sargent, M. V.; Wangchareontrakul, S.; *J. Chem. Soc. Perkin I.* **1989**, 1171-1172.

[186] Erickson, J.; Schott, D.; Reverri, T.; Muhsin, W.; Ruttledge, T.; *J. Agric. Food Chem.* **2001**, *49*, 5537-5542.

[187] Bouillant, M. L.; Jacoud, C.; Zanella, I.; Favrebonvin, J.; Bally, R.; *Phytochemistry* **1994**, *35*, 769-771.

[188] Suzuki, Y.; Esumi, Y.; Hyakutake, H.; Kono, Y.; Sakurai, A.; *Phytochemistry* **1996**, *41*, 1485-1489.

[189] Suzuki, Y.; Esumi, Y.; Saito, T.; Kishimoto, Y.; Morita, T.; Koshino, H.; Uzawa, J.; Kono, Y.; Yamaguchi, I.; *Phytochemistry* **1998**, *47*, 1247-1252.

[190] Kubus, G.; Tluscik, F.; *Acta Soc. Bot. Polon.* **1983**, *53*, 223 - 230.

[191] Metzger, P.; *Phytochemistry* **1994**, *36*, 195-212.

[192] Zarnowski, R.; Suzuki, Y.; Esumi, Y.; Pietr, S. J.; *Phytochemistry* **2000**, *55*, 975-977.

[193] Gregson, R. P.; Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; *Aust. J. Chem.* **1977**, *30*, 2527 - 2532.

[194] Stodola, F. H.; Weisleder, D.; Vesonder, R. F.; *Phytochemistry* **1973**, *12*, 1797 - 1798.

[195] Voblikova, V. D.; Kobrina, N. S.; Gerasimova, N. M.; Pavlova, Z. N.; Dem'yanova, G. F.; Murygina, V. P.; Volosova, L. I.; Muromtsev, G. S.; *Khim. Prir. Soedin.* **1985**, 387 - 391.

[196] Deffieux, G.; Baute, R.; Bourgeois, G.; Neveu, A.; Filleau, M. J.; *Bull. Soc. Pharm. Ouest.* **1974**, *16*, 259 - 263.

[197] Zarnowski, R.; Lewicka, T.; Pietr, S. J.; *Z. Naturforsch. C* **2000**, *55c*, 846-848.
[198] Ayer, W. A.; Browne, L. M.; Lovell, S. H.; *Phytochemistry* **1983**, *22*, 2267 - 2271.
[199] Ding, Z. W.; Dong, Z. J.; Liu, J. K.; *Helv. Chim. Acta* **2001**, *84*, 259-262.
[200] Kumagai, M.; Suhara, Y.; Aoyagi, T.; Umezawa, H.; *J. Antibiot.* **1971**, *24*, 870 - 875.
[201] Tsuge, N.; Mizokami, M.; Imai, S.; Shimazu, A.; Seto, H.; *J. Antibiot.* **1992**, *45*, 886-891.
[202] Yang, Q.; Asai, M.; Yoshihara, T.; *Z. Naturforsch. C* **2000**, *55c*, 546-551.
[203] Barr, J. R.; Murty, V. S.; Yamaguchi, K.; Shangara, S.; Smith, D. H.; *Chem. Res. Toxicol.* **1988**, *1*, 204 - 207.
[204] Suresh, M.; Ray, R. K.; *Curr. Sci.* **1990**, *59*, 477 - 479.
[205] Crowder, J. A.; Stodola, F. H.; Anderson, R. J.; *J. Biol. Chem.* **1936**, *114*, 431 - 435.
[206] Yamada, H.; Shiomi, K.; Xu, Q.; Nagai, T.; Shibata, M.; Oya, I.; Takahashi, Y.; Omura, S.; *J. Antibiot.* **1995**, *48*, 205-210.
[207] Gellerman, J. L.; Anderson, W. H.; Schlenk, H.; *Biochim. Biophys. Acta* **1976**, *431*, 16-21.
[208] Sargent, M. V.; Wangchareontrakul, S.; Jefferson, S.; *J. Chem. Soc. Perkin I.* **1989**, 431.
[209] Canedo, L. M.; Delcorral, J. M. M.; Sanfeliciano, A.; *Phytochemistry* **1997**, *44*, 1559-1563.
[210] Furstner, A.; Seidel, G.; *J. Org. Chem.* **1997**, *62*, 2332-2336.
[211] Zarnowski, R.; Zarnowska, E. D.; Kozubek, A.; *Acta Soc. Bot. Polon.* **2001**, *70*, 25-29.
[212] He, J.; Xie, B.; *J. Chromatogr. A* **2002**, *943*, 303-309.
[213] He, J. R.; Xie, B. J.; *Acta Chim. Sinica* **2001**, *59*, 1153-1157.
[214] Van Beek, T. A.; Wintermans, M. S.; *J. Chromatogr. A* **2001**, *930*, 109-117.
[215] Van Beek, T. A.; *J. Chromatogr. A* **2002**, *967*, 21-55.
[216] Ndjoko, K.; Wolfender, J.-L.; Hostettmann, K.; *J. Chromatogr. B* **2000**, *744*, 249-255.
[217] Kamiya, Y.; Saito, W.; Miyakoshi, T.; *J. Oleo. Sci.* **2002**, *51*, 473-483.
[218] Yang, L.-K.; Khoo-Beatttie, C.; Goh, K.-L.; Chng, B.-L.; Yoganathan, K.; Lai, Y.-H.; Butler, M. S.; *Phytochemistry* **2002**, *58*, 1235-1238.
[219] Shobha, S. V.; Ravindranath, B.; *J. Agric. Food Chem.* **1991**, *39*, 2214-2217.
[220] Bartle, K. D.; Clifford, A. A. In *Developments in the Analysis of Lipids*; Tyman, J. H. P., Ed.; Royal Soc. Chem.: Cambridge, **1994**.
[221] Tyman, J. H. P.; Bruce, I. E.; Payne, P.; *Nat. Prod. Lett.* **1992**, *1*, 117-120.
[222] Tyman, J. H. P. In *Quantitative Thin-layer Chromatography and its Industrial Applications*; Laszlo, L. R., Ed., **1987**; Vol. 36.
[223] Nagabhushana, K. S.; B., R.; *J. Agric. Food Chem.* **1995**, *43*, 2381-2383.
[224] Caplin, J.; Tyman, J. H. P.; *J. Chem. Res.* **1982**, (S) 34-35, (M) 0321-0351.
[225] Baylis, C. J.; Odle, S. W. D.; J.H.P., T.; *J. Chem. Soc. Perkin I* **1981**, 132-141.
[226] Tyman, J. H. P.; Schofield, B. G.; Khor, C. H.; *Chem. Phys. Lipids* **2002**, *120*, 101-108.
[227] Tyman, J. H. P.; Khor, C. H.; *Chem. Ind.* **1974**, 526-527.

[228] Alonso, E.; Ramon, D. J.; Yus, M.; *J. Org. Chem.* **1997**, *62*, 417-421.
[229] Brown, H. C.; Campbell, J., J.B.; *J. Org. Chem.* **1980**, *45*, 389-395.
[230] Miyakoshi, T.; Kobuchi, H.; *Nippon Kagaku Kaishi* **1990**, 1271.
[231] Miyakoshi, T.; Kobuchi, H.; Niimura, N.; Yoshimoto, Y.; *Bull. Chem. Soc. Jpn.* **1991**, 2562.
[232] Niimura, N.; Kamiya, Y.; Sato, T.; Katano, I.; Miyakoshi, T.; *J. Jpn. Oil Chem. Soc.* **1998**, *47*, 171-178.
[233] Kamiya, Y.; Miyakoshi, T.; *J. Oleo Sci.* **2001**, *50*, 865-874.
[234] Green, I. R.; Tocoli, F. E.; *Synt. Commun.* **2002**, *32*, 947-957.
[235] Durrani, A. A.; Tyman, J. H. P.; *J. Chem. Soc. Perkin I* **1980**, 1658-1666.
[236] Tyman, J. H. P.; Visani, N.; *J. Chem. Research.(S)* **1997**, (S) 14-15, (M) 0228-0240.
[237] Harris, T. M.; Hill, J. E.; *Synth. Commun.* **1982**, *12*, 621.
[238] Bruce, I.; Spencer, R.; Tyman, J. H. P.; *J. Chem. Res.* **1992**, (S) 224-225, (M) 1773-1795.
[239] Satoh, M.; Takeuchi, N.; Fujita, T.; Yamazaki, K.; Tobinaga, S.; *Chem. Pharm. Bull.* **1998**, *46*, 1501-1505.
[240] Satoh, M.; Takeuchi, N.; Nishimura, T.; Ohta, T.; Tobinaga, S.; *Chem. Pharm. Bull.* **2001**, *49*, 18-22.
[241] Zehnter, R.; Gerlach, H.; *Liebigs Annalen* **1995**, 2209-2220.
[242] Tyman, J. H. P.; *J. Org. Chem.* **1976**, *41*, 894.
[243] Kamiya, Y.; Miyakoshi, T.; *Polymer Preprints, Jpn.* **1999**, *48*, II MO9.
[244] Yurukawa, Y.; Yamagiwa, Y.; Kamikawa, T.; *J. Chem. Soc. Chem. Commun.* **1986**, 1234.
[245] Sollardie, G.; Ziani-Cherif, C.; *J. Org. Chem.* **1993**, *58*, 2181.
[246] In *The Cashew Export Promotion Council, Cashew Nushell Liquid Patents*; India: Ernakulam-6, **1964**; Vol. I and II.
[247] Harvey, M. T.; *US Patent 2368709* **1941**.
[248] Stadeler, A. J.; *Ann. Chim. Pharm.* **1847**, *63*, 137.
[249] Minnikin, D. E.; Barer, M. R.; Gernaey, A. M.; Garton, J. G.; Colvine, J. R. L.; Douglas, J. D.; Ahmed, A. M. S. In *Lipids in Health and Nutrition*; Tyman, J. H. P., Ed.; Royal Society of Chemistry: Cambridge, **1999**.
[250] Mizushina, Y.; Murakami, C.; Ohta, K.; Takikawa, H.; Mori, K.; Yoshida, H.; Sugawara, F.; Sakaguchi, K.; *Biochem. Pharmacol.* **2002**, *63*, 399-407.
[251] Johnson, M. K.; Alexander, K. E.; Lindquist, N.; Loo, G.; *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology* **1999**, *122*, 211-214.
[252] Amorati, R.; Attanasi, O. A.; El Ali, B.; Filippone, P.; Mele, G.; Spadavecchia, J.; Vasapollo, G.; *Synthesis-Stuttgart* **2002**, 2749-2755.
[253] Patel, M. *Ph.D. Thesis*; Brunel University: London, **1979**.
[254] Tyman, J. H. P. In *Handbook of thin layer chromatography*; Sherma, J., Fried, B., Eds.; Marcel Dekker: New York, **2003**.
[255] Stahl, E.; Keller, K.; Blinn, C.; *Planta Med.* **1983**, *48*, 5 - 9.
[256] Adawadkar, P. D.; Elsohly, M. A.; *Fitoterapia* **1981**, *52*, 129 - 135.
[257] Shimomura, K.; Koizumi, Y.; *JP Patent 93,240,718* **1991**.
[258] Muroi, H.; Kubo, A.; Kubo, I.; *J. Agric. Food Chem.* **1993**, *41*, 1106-1109.

[259] Muroi, H.; Kubo, I.; *Biosci. Biotech. Biochem.* **1994**, *58*, 1925-1926.
[260] Muroi, H.; Kubo, I.; *J. Appl. Bacteriol.* **1996**, *80*, 387-394.
[261] Muroi, H.; Kubo, I.; *J. Agric. Food Chem.* **1993**, *41*, 1780-1783.
[262] Kubo, I.; Sukigara, M.; *PCT Intl. Appl., WO 95 30,417* **1995**.
[263] Kubo, I.; Muroi, H.; Himejiwa, M.; Yamagiva, Y.; Mera, H.; Tokushima, K.; Ohta, S.; Kamikawa, T.; *J. Agric. Food Chem.* **1993**, *41*, 1016-1019.
[264] Kuwata, K.; Konishi, M.; *JP 09,214,675* **1996**.
[265] Kuwata, K.; Konishi, M.; *JP 10,029,918* **1998**.
[266] Kuwata, K.; Konishi, M.; *JP 10,036,244* **1998**.
[267] Kuwata, K.; Konishi, M.; *JP 10,036,883* **1998**.
[268] Kuwata, K.; Konishi, M.; *JP 10,036,884* **1998**.
[269] Kuwata, K.; Onishi, M. In *Jpn. Kokai Tokkyo Koho* Japan, **1998**.
[270] Izume, M.; Tajima, M.; *JP. 1036, 214* **1998**.
[271] Kubo, J.; Lee, J. R.; Kubo, I.; *Journal of Agricultural and Food Chemistry* **1999**, *47*, 533 - 537.
[272] Molyneux, R. J.; Maloney, N.; Campbell, B. C. In *ACS Symposium Series 745*; Tu, A. T., Garfield, W., Eds.
[273] Laurens, A.; Fourneau, C.; Hocquemiller, R.; Cave, A.; Bories, C.; Loiseau, P. M.; *Phytotherapy Research* **1997**, *11*, 145-146.
[274] Hammond, D.; Kubo, I.; *US Patent 6,077,521* **2000**.
[275] Subba Rao, P. V.; Kumble, S. P.; Annadurai, R. S.; Srinivas, M.; Rao, A. S.; Ramadoss, C. S.; *PCT Intl. Appl. WO 00 54,596* **2000**.
[276] Lloyd, H. A.; Denny, C.; Krishna, G.; *J. Liquid Chromatogr.* **1980**, *3*, 1497-1502.
[277] Mendes, N. M.; De Oliveira, A. B.; Guimaraes, J. E.; Pereira, J. P.; Katz, N.; *Rev. Soc. Bras. Med. Trop.* **1990**, *23*, 217 - 224.
[278] Hird, N. W.; Milner, P. H.; *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1423-1428.
[279] Arogba, S. S. *Ph.D. Thesis*; Brunel University: London, **1987**.
[280] Irie, J.; Murata, M.; Homma, S.; *Biosci. Biotechnol. Biochem.* **1996**, *60*, 240-243.
[281] Tyman, J. H. P. In *Lipids in Health and Nutrition*; Tyman, J. H. P., Ed.; Royal Society of Chemistry: Cambridge, **1999**.
[282] Murata, M.; Irie, J.; Homma, S.; *Lebens.-Wiss. u. Technol.* **1997**, *30*, 458-463.
[283] Toyomizu, M.; Sugiyama, S.; Jin, R. L.; Nakatsu, T.; *Phytother. Res.* **1993**, *7*, 252-254.
[284] Wang, D. D.; Girard, T. J.; Kasten, T. P.; Lachance, R. M.; Millerwideman, M. A.; Durley, R. C.; *J. Nat. Prod.* **1998**, *61*, 1352-1355.
[285] Kuo, S.-C.; Teng, C.-M.; Lee, L.-G.; Chiu, T.-H.; Wu, T.-S.; Huang, S.-C.; Wu, J.-B.; Shieh, T.-Y.; Chang, R.-J.; Chou, T.-C.; *Planta Med.* **1991**, *57*, 247 - 249.
[286] Kanojia, R. M.; Murray, W.; Bernstein, J.; Fernandez, J.; Foleno, J.; Krause, H.; Lawrence, L.; Webb, G.; Barrett, J. F.; *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2947 - 2952.
[287] Paramashivappa, R.; Kumar, P. P.; Rao, P. V. S.; Rao, A. S.; *Journal of Agricultural and Food Chemistry* **2002**, *50*, 7709-7713.
[288] Shobha, S. V.; Ramadoss, C. S.; Ravindranath, B.; *J. Nat. Prod.* **1992**, *55*, 818.

[289] Shobha, S. V.; Ramadoss, C. S.; Ravindranath, B.; *J. Nat. Prod.* **1994**, *57*, 1755-1757.

[290] Hesk, D.; Craig, R.; Mumma, R.; *J. Chem. Ecol.* **1992**, *18*, 1349 - 1353.

[291] Craig, R.; Medford, J.; Mumma, R. O.; Cox-Foster, D.; Schultz, D.; *US Patent 5,856,157* **1999**.

[292] Paramashivappa, R.; Phani Kumar, P. P.; Subba Rao, P. V. S.; Srinivasa Rao, A. S.; *Bioorg. Med. Chem. Lett.* **2003**, *13*, 657-660.

[293] Hecker, H.; Johannisson, R.; Koch, E.; Siegers, C. P.; *Toxicology* **2002**, *177*, 167-177.

[294] Toyomizu, M.; Okamoto, K.; Ishibashi, T.; Chen, Z. Q.; Nakatsu, T.; *Life Sci.* **2000**, *66*, 229 - 234.

[295] Toyomizu, M.; Okamoto, K.; Akiba, Y.; Nakatsu, T.; Konishi, T.; *Biochim. Biophys. Acta* **2002**, *1558*, 54-62.

[296] He, W.; Puyvelde, L. V.; Bosselaers, J.; De Kimpe, N.; Van Den Flaas, M.; Roymans, A.; Mathenge, S. G.; Mutida, F. P.; Mutiso, P. B. C.; *Pharm. Biol.* **2002**, *40*, 74-76.

[297] Lepoittevin, J.; Benezra, C.; Asakawa, Y.; *Arch Dermatol Res* **1989**, *281*, 227-30.

[298] Klarmann, E.; *J. Am. Chem. Soc.* **1926**, *48*, 2358 - 2367.

[299] Klarmann, E.; Gatyas, L. W.; Shternov, V. A.; *J. Am. Chem. Soc.* **1932**, *54*, 298 - 305.

[300] Klarmann, E.; Gatyas, L. W.; Shternov, V. A.; *J. Am. Chem. Soc.* **1932**, *54*, 1204 - 1211.

[301] Klarmann, E.; Gatyas, L. W.; Shternov, V. A.; *J. Am. Chem. Soc.* **1931**, *53*, 3397 - 3407.

[302] Leonard, V.; Wood, A.; *J. Am. Med. Assoc.* **1925**, *85*, 1855 - 1859.

[303] Schaffer, J. M.; Tilley, F. W.; *J. Bacteriol.* **1927**, *14*, 259 - 273.

[304] Stapp, C.; *Agnew Bot.* **1930**, *12*, 275 - 289.

[305] Erin, A. N.; Davitashvili, N. G.; Prilipko, L. L.; Boldyrev, A. A.; Lushchak, V. I.; Batrakov, S. G.; Pridachina, N. N.; Serbinova, A. E.; Kagan, V. E.; *Biokhimiya* **1987**, *52*, 1180 - 1185.

[306] Kaprelyants, A. S.; Suleimenov, M. K.; Sorokina, A. D.; Deborin, G. A.; El-Registan, G. I.; Stoyanovich, F. M.; Lille, Y. E.; Ostrovsky, D. N.; *Biol. Membr.* **1987**, *4*, 254 - 261.

[307] Himejima, M.; Kubo, I.; *J. Agric. Food Chem.* **1991**, *39*, 418 - 421.

[308] Kraal, J. H.; Hussain, A. A.; Gregorio, S. B.; Akaho, E.; *J. Dent. Res.* **1979**, *58*, 2125 - 2131.

[309] Gosteli, J.; *Ger. Offen. 2,359,410* **1974**.

[310] Henderson, E.; *Brit. Patent 1,186,691* **1970**.

[311] Dohme, A. R. L.; *U.S. Patent 1,858,042* **1931**.

[312] Katsuta, T.; *Patent Jpn. Kokai Tokkyo Koho J.P. 02.255.609* **1990**.

[313] Riedel, J. D. E.; De Haen, A. G.; *Patent Ger., 555,291* **1929**.

[314] Uchida, H.; *Jpn. Kokai Tokkyo Koho J.P. 60,139,637* **1985**.

[315] Droby, S.; Prusky, D.; Jacoby, B.; Goldman, A.; *Physiol. Mol. Plant Pathol.* **1987**, *30*, 285 - 292.

[316] Reiss, J.; *Cereal Chem.* **1989**, *66*, 491 - 493.

[317] Garcia, S.; Garcia, C.; Heinzen, H.; Moyna, P.; *Phytochemistry* **1997**, *44*, 415-418.

[318] Kubo, I.; Komatsu, S.; Ochi, M.; *J. Agric Food Chem.* **1986**, *34*, 970 - 973.

[319] Nahas, G. G.; Desoize, B.; *Hebd. Seances Acad. Sci., Ser. D* **1974**, *279*, 1607 - 1608.

[320] Ogiu, K.; Yamada, H.; Shirasaki, K.; *Japan J. Pharm. Chem.* **1950**, *22*, 355 - 357.

[321] Ogiu, K.; Yamada, H.; Tsukamoto, T.; Kobayashi, M.; Sawada, M.; *Folia Pharmacol. Japon.* **1950**, *45*, 106 - 110.

[322] Itokawa, H.; Totsuka, N.; Nakahara, K.; Maezuru, M.; Takeya, K.; Kondo, M.; Inamatsu, M.; Morita, H.; *Chem. Pharm. Bull.* **1989**, *37*, 1619 - 1621.

[323] Itokawa, H.; Totsuka, N.; Nakahara, K.; Takeya, K.; Lepoittevin, J.-P.; Asakawa, Y.; *Chem. Pharm. Bull.* **1987**, *35*, 3016 - 3020.

[324] Kozubek, A.; *Acta Biochim. Polon.* **1987**, *34*, 387 - 394.

[325] Tamai, H.; Yamane, T.; Tasaka, K.; *Patent Jpn. Kokai Tokkyo Koho J.P.02.292.213* **1990**.

[326] Matsumoto, K.; Fujimoto, M.; Ito, K.; Tanaka, H.; Hirondo, I.; *J. Toxicol. Sci.* **1990**, *15*, 39 - 46.

[327] Suzuki, Y.; Yamaguchi, I.; *Nippon Noyaku Gakkaishi* **1998**, *23*, 316-321.

[328] Filip, P.; Anke, T.; Sterner, O.; *Z. Naturforsch. C* **2002**, *57c*, 1004-1008.

[329] Sedlet, K.; Mathias, M.; Lorenz, K.; *Cereal Chem.* **1984**, *61*, 239 - 241.

[330] Harumoto, T.; Miyake, A.; Ishikawa, N.; Sugibayashi, R.; Zenfuku, K.; Iio, H.; *Eur. J. Protistol.* **1998**, *34*, 458-470.

[331] Zarnowski, R.; Pietr, S. J.; Hendrich, A. Third Int. Symp. on Rhizoctonia, Taichung, Taiwan, ROC, **2000**.

[332] Zarnowski, R.; Kozubek, A.; Pietr, S.; *Bull. Pol. Acad. Sci.* **1999**, *47*, 231 - 235.

[333] Komolova, G. S.; Gorskaya, I. A.; Kaverinskaya, T. V.; Sheveleva, I. D.; *Biokhimiya* **1989**, *54*, 1847 - 1851.

[334] Hecht, S. M.; *Pure Appl. Chem.* **1989**, *61*, 577 - 580.

[335] Lytollis, W.; Scannell, R. T.; An, H. Y.; Murty, V. S.; Reddy, K. S.; Barr, J. R.; Hecht, S. M.; *J. Am. Chem. Soc.* **1995**, *117*, 12683-12690.

[336] Singh, U. S.; Scannell, R. T.; An, H.; Carter, B. J.; Hecht, S. M.; *J. Am. Chem. Soc.* **1995**, *117*, 12691-12699.

[337] Tyman, J. H. P.; Matthews, A. J.; *J. Chromatogr.* **1982**, *235*, 149-154.

[338] He, S.; *Shengwu Huaxue Zazhi* **1990**, *6*, 428 - 431.

[339] Kozasa, T.; Yamamoto, H.; Tsunoda, N.; *Patent Jpn. Kokai Tokkyo Koho J.P. 02.101.036* **1990**.

[340] Starck, S. R.; Deng, J. Z.; Hecht, S. M.; *Biochemistry* **2000**, *39*, 2413-2419.

[341] Deng, J. Z.; Marshall, R.; Jones, S. H.; Johnson, R. K.; Hecht, S. M.; *Journal of Natural Products* **2002**, *65*, 1930-1932.

[342] Kozubek, A.; Nienartowicz, B.; *Acta Biochim. Polon.* **1995**, *42*, 309-316.

[343] Adam, N. K.; *Proc. Roy. Soc. (Ser.A)* **1928**, *119*, 628 - 644.

[344] Kozubek, A.; *Acta Univ. Wratisl.* **1986**, *886*, 122pp.

[345] Sikorski, A. F.; Michalak, K.; Bobrowska, M.; Kozubek, A.; *Stud. Biophys.* **1987**, *121*, 183 - 191.

[346] Kieleczawa, J.; Szalewicz, A.; Kozubek, A.; Kulig, E.; *Progr. Photosynth. Res.* **1987**, *2*, 585 - 587.
[347] Kozubek, A.; *Acta Biochim. Polon.* **1995**, *42*, 241-246.
[348] Kozubek, A.; Nietubyc, M.; Sikorski, A. F.; *Z. Naturforsch.* **1992**, *47c*, 41-46.
[349] Kozubek, A.; Wroblewski, Z.; *Stud. Biophys.* **1990**, *139*, 177 - 181.
[350] Kozubek, A.; *Z. Naturforsch.* **1992**, *47c*, 608-612.
[351] Gordeev, K. Y.; Bitkov, V. V.; Pridachina, N. N.; Nenashev, V. A.; Batrakov, S. G.; *Bioorg. Khim.* **1991**, *17*, 1357 - 1364.
[352] Rejman, J.; Kozubek, A.; *Cell. Mol. Biol. Lett.* **1997**, *2*, 411-419.
[353] Vincieri, F. F.; Vinzenzini, M. T.; Vanni, P.; *Riv. Ital. EPPOS* **1981**, *63*, 79 - 82.
[354] Kozubek, A.; *Acta Univ. Wratisl.* **1989**, *868*, 27 - 32.
[355] Nenashev, V. A.; Pridachina, N. N.; Elregistan, G. I.; Zolotareva, I. N.; Batrakov, S. G.; *Biokhimia* **1994**, *59*, 11-15.
[356] Nenashev, V. A.; Pridachina, N. N.; Pronevich, L. A.; Batrakov, S. G.; *Biokhimiya* **1989**, *54*, 784 - 787.
[357] Roufogalis, B. D.; Li, Q.; Tran, V. H.; Kable, E. P. W.; Duke, C. C.; *Drug Development Research* **1999**, *46*, 235.
[358] Kinoshita, K.; Saito, D.; Koyama, K.; Takahashi, K.; Sato, Y.; Okuyama, E.; Fujimoto, H.; Yamazaki, M.; *Journal of the Hattori Botanical Laboratory* **2002**, 277-284.
[359] Mechoulam, R.; Hanus, L.; *Chem. Phys. Lipids* **2000**, *108*, 1-13.
[360] Lepoittevin, J.; Benezra, C.; *J Med Chem* **1986**, *29*, 287-91.
[361] Lepoittevin, J.-P.; Benezra, C.; *Pharm. Weekblad Sci. Ed.* **1991**, *13*, 119 - 122.
[362] 'T Hart, L. A.; *Pharmaceutische Weekblad Scientific Edition* **1991**, *13*, 127 - 129.
[363] Diogenes, M. J. N.; Demorais, S. M.; Carvalho, F.; *Int. J. Dermatol.* **1995**, *34*, 72-73.
[364] Diogenes, M. J. N.; Demorais, S. M.; Carvalho, F. F.; *Contact Dermatitis* **1996**, *35*, 114-115.
[365] Knight, T. E.; *Cutis* **1991**, *48*, 375-378.
[366] Knight, T. E.; Boll, P.; Epstein, W. L.; Prasad, A. K.; *Am. J. Contact Dermat.* **1996**, *7*, 138-145.
[367] Menz, J.; Rossi, E. R.; Taylor, W. C.; Wall, L.; *Contact Dermatitis* **1986**, *15*, 126-131.
[368] Baer, H.; Watkins, R. C.; Kurtz, A. P.; Byck, J. S.; Dawson, C. R.; *J. Immunol.* **1967**, *99*, 365 - 369.
[369] Benezra, C.; *Prog Clin Biol Res* **1988**, *280*, 395-400.
[370] Kalish, R. S.; Wood, J. A.; Laporte, A.; *J. Clin. Investig.* **1994**, *93*, 2039-2047.
[371] Kalergis, A. M.; Lopez, C. B.; Becker, M. I.; Diaz, M. I.; Sein, J.; Garbarino, J. A.; Deioannes, A. E.; *J. Invest. Dermatol.* **1997**, *108*, 57-61.
[372] Kozubek, A.; *Acta Biochim. Polon.* **1995**, *42*, 247-252.
[373] Kozubek, A.; Demel, R. A.; *Biochim. Biophys. Acta* **1981**, *642*, 242 - 251.
[374] Hendrich, A. B.; Kozubek, A.; *Z. Naturforsch.* **1991**, *46c*, 423 - 427.
[375] Kozubek, A.; *Post. Biochem.* **1993**, *39*, 264-270.
[376] Gerdon, S.; Hoffmann, S.; Blume, A.; *Chem. Phys. Lipids* **1994**, *71*, 229-243.

[377] Kozubek, A.; Jezierski, A.; Sikorski, A. F.; *Biochim. Biophys. Acta* **1988**, *944*, 465 - 472.
[378] Bitkov, V. V.; Nenashev, V. A.; Khashaev, Z. H.; Pridachina, N. N.; Batrakov, S. G.; *Biol. Membr.* **1990**, *7*, 135 - 140.
[379] Bitkov, V. V.; Nenashev, V. A.; Khashaev, Z. K.; Pridachina, N. N.; Shishlov, Y. V.; Batrakov, S. G.; *Biol. Membr.* **1988**, *5*, 1055 - 1060.
[380] Bitkov, V. V.; Nenashev, V. A.; Pridachina, N. N.; Batrakov, S. G.; *Biochim. Biophys. Acta* **1992**, *1108*, 224-232.
[381] Przeworska, E.; Gubernator, J.; Kozubek, A.; *Biochim. Biophys. Acta* **2001**, *1513*, 75-81.
[382] Przeworska, E.; Gubernator, J.; Kozubek, A. W. Mejbaum-Katzenellenbogen's Seminars in Molecular Biology. 3. Liposomes in Biology and Medicine, Wroclaw, **1996**; p P15.
[383] Kozubek, A.; Demel, R. A.; *Biochim. Biophys. Acta* **1980**, *603*, 220 - 227.
[384] Kozubek, A.; Skala, J.; *Z. Naturforsch.* **1995**, *50c*, 656-659.
[385] Kozubek, A.; *Acta Biochim. Polon.* **1987**, *34*, 357 - 367.
[386] Kozubek, A.; *Z. Naturforsch.* **1985**, *40c*, 80 - 84.
[387] Kozubek, A.; *Z. Naturforsch.* **1984**, *39c*, 1132 - 1136.
[388] Stasiuk, M.; Kozubek, A.; *Cell. Mol. Biol. Lett.* **1997**, *2*, 77 - 87.
[389] Stasiuk, M.; Kozubek, A.; *Cell. Mol. Biol. Lett.* **1996**, *1*, 189-198.
[390] Yamamoto, S.; Nakano, K.; Ishikawa, C.; Yamamoto, M.; Matsumoto, Y.; Iwahara, M.; Furusaki, S.; Ueoka, R.; *Biochem. Engin. J.* **2002**, *12*, 125-130.
[391] Gubernator, J.; Kozubek, A.; *Chem. Phys. Lipids* **1998**, *94*, 177.
[392] Gubernator, J.; Stasiuk, M.; Kozubek, A.; *Biochim. Biophys. Acta* **1999**, *1418*, 253-260.
[393] Gubernator, J.; Kozubek, A.; Grynkiewicz, G. Polska, **1998**.
[394] Gubernator, J.; Kozubek, A.; Grynkiewicz, G. Polska, **1998**.
[395] Gubernator, J.; Kozubek, A. Polska, **1998**.
[396] Kozubek, A.; Gubernator, J.; Przeworska, E.; Stasiuk, M.; *Acta Biochim. Polon.* **2000**, *47*, 639-649.
[397] Struski, D. G. J.; Kozubek, A.; *Z. Naturforsch.* **1992**, *47c*, 47-50.
[398] Nienartowicz, B.; Kozubek, A.; *Pol. J. Food Nutr. Sci.* **1993**, *2*, 51-60.
[399] Winata, A.; Lorenz, K.; *J. Food Process. Preservat.* **1996**, *20*, 417-429.
[400] Zubik, L.; Hladyszowski, J.; Czucha, B.; Kozubek, A. W. Mejbaum-Katzenellenbogen's Seminars in Molecular Biology. 3. Liposomes in Biology and Medicine, Wroclaw, **1996**; p P24.
[401] Kozubek, A. A. 10th European Union Young Scientsists Contest, Porto, Portugal, **1998**; p 37.
[402] Hladyszowski, J.; Zubik, L.; Kozubek, A.; *Free Radical Res.* **1998**, *28*, 359 - 368.
[403] Kamal-Eldin, A.; Pouru, A.; Eliasson, C.; Aman, P.; *J. Sci. Food. Agric.* **2001**, *81*, 353-356.
[404] Wagner, H.; Breu, W.; Willer, F.; Wierer, M.; Remiger, P.; Schwenker, G.; *Planta Med.* **1989**, *55*, 566 - 567.
[405] Wagner, H.; Wierer, M.; Bauer, R.; *Planta Med.* **1986**, 184 - 187.
[406] Deszcz, L.; Kozubek, A.; *Cell. Mol. Biol. Lett.* **1997**, *2*, 213 - 222.

[407] Shobha, S. V.; Ramadoss, C. S.; Ravindranath, B.; *J. Nat. Prod.* **1994**, *57*, 1755-1757.
[408] Hengtrakul, P.; Mathias, M.; Lorenz, K.; *J. Nutr. Biochem.* **1991**, *2*, 20 - 24.
[409] George, J.; Kuttan, R.; *Cancer Lett* **1997**, *112*, 11-16.
[410] Gasiorowski, K.; Brokos, B.; Kozubek, A.; Oszmianski, J.; *Cell. Mol. Biol. Lett.* **2000**, *5*, 171-190.
[411] Gasiorowski, K.; Szyba, K.; Brokos, B.; Kozubek, A.; *Cancer Lett.* **1996**, *106*, 109-115.
[412] Ross, A. B.; *Acta Univ. Agric. Suec.* **2003**, *Agraria 417*, 1-132.
[413] Ross, A. B.; Chen, Y; Frank, J.; Swanson, J.E.; Parker, R.S.; Kozubek, A.; Lund, T.; Vessby, B.; Aman,P.; Kamal-Eldin,A.; *J. Nutrit.* **2004**, *134*, 506-510.
[414] Ross, A. B. ; Kamal-Eldin,A; Lundin, E.A.; Zhang, J-X.; Halmans.G.; Aman.P.; *J. Nutrit.* **2003**, 2222-2224.
[415] Ross, A. B.; Shepherd, M.J.; Knudsen, K.E. B.; Glitz,L.V.; Bowey, E.; Phillips, J.; Rowland, I.; Guo, Z. X.; Massy, D. J. R.; Aman. P.; Kamal-Eldin, A.; *Brit.* J. Nutrit. **2003**, *90*, 787-794.
[416] Linko, A. M.; Parikka, K.; Wahala, K.; Adlercreutz, H.; *Anal. Biochem.* **2002**, *308,* 307-313.
[417] Rejman, J.; Kozubek, A.; *J.Agric. Food Chem.* **2004**, *52,* 246-250.
[418] Ross, A.B.; Kamal-Eldin,A.; Aman, P.; *Nutrit. Rev.*, **2004**, *62*, 81-95

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# THE USE OF NATURAL PRODUCTS AS SOURCES OF NEW ANALGESIC DRUGS

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**ABSTRACT:** Natural substances derived from plants have played an extremely important role in the development of analgesic drugs and in the understanding of the complex mechanisms involved in pain transmission and pain relief. Salicin, a glycoside obtained from the bark of *Salix* species, was the lead compound for the synthesis of aspirin based on its activity and structural properties. Aspirin has contributed to the development of a group of drugs to treat pain and inflammation and more importantly to the comprehension at the molecular level of relevant pain mechanisms. As a consequence, a new generation of cyclo-oxygenase inhibitors has been recently developed. Other studies have contributed to the determination and reproduction of some important receptors for morphine, $\Delta^9$-tetrahydrocannabinol, and capsaicin. These are important and established analgesic compounds which show undesirable side effects. Such findings have opened new possibilities for research into new potent analgesic drugs based on structure-activity relationships. Recent advances regarding plant-derived compounds with antinociceptive action are also discussed herein along with insights into their structure-activity relationships.

## INTRODUCTION

Natural products are fundamental to pain treatment. They yield new analgesics and play an important role in the study of pain mechanisms [for review see 1,2]. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Alternatively, it can be described in terms of such damage [3]. The sensory component of pain (nociception) is triggered by mechanical, thermal, or chemical noxious stimuli at the free ends of small diameter primary afferent fibres (nociceptors) present in practically all body tissues [4,5]. After tissue damage, several mediators, such as bradykinin,

histamine, prostaglandins, leukotrienes, cytokines, noradrenaline, substance P, and glutamate, among others, are released from vessels, mast cells, macrophages, neutrophils, as well as sympathetic and afferent fibres producing nociceptor activation and sensitisation of other noxious stimuli [6]. The importance of the production of a mediator for pain induction may be realized from the large and effective clinical use of prostaglandin inhibitors (non-steroidal anti-inflammatory drugs - NSAIDs) for the treatment of painful conditions including rheumatism, osteoarthritis, headache, and dental surgery [7].

## PLANT COMPOUNDS THAT HAVE CONTRIBUTED TO THE DEVELOPMENT OF ANALGESIC DRUGS

The prototype and first NSAID produced was aspirin (acetylsalicylic acid) synthesised from willow-derived salicylic acid. Although willow (*Salix* species) has long been used in traditional medicine for its antipyretic, anti-inflammatory and analgesic properties, only in the 70s' was aspirin discovered to inhibit the cyclooxygenase enzyme (COX), which is responsible for the formation of prostanoids from arachidonic acid [8,9]. Further studies revealed the existence of two isoforms of COX, named 1 and 2. More recently, the presence of a new COX enzyme in the brain (COX3) was reported [10,11]. All three COX enzymes are inhibited by classical NSAIDs such as aspirin, indomethacin and ibuprofen. With a few exceptions, COX-2 is normally induced during inflammation, producing pro-nociceptive prostaglandins. On the other hand, COX-1 is constitutively expressed in some tissues, such as stomach and kidney, where it is involved in physiological effects. Thus, classical NSAIDs produce serious undesirable side effects, such as gastrointestinal and renal disorders. Furthermore, new selective and less toxic NSAIDs, known as COX-2 inhibitors (e.g., rofecoxib and celecoxib), have been developed and launched on the market in recent years to treat inflammatory and pain disorders [10]. COX-3 was recently cloned and it is selectively inhibited by analgesics/antipyretics such as acetaminophen and dipyrone [11]. Prostaglandins produce their nociceptive effect by activating $G\alpha s$-coupled prostanoid receptors in sensory neurones [12]. An increase in cAMP levels and kinase A protein (PKA) stimulation regulates several ionotropic receptors involved in nociceptor depolarisation including tetrodotoxin-resistant sodium

channels and vanilloid receptors (transient receptor potentials, TRPV1) [13,14].

TRPV1 (formerly called VR1) is endogenously activated by noxious heat, acidosis, and lipid mediators [15]. It was discovered through its selective sensitivity to the naturally-occurring substance capsaicin (trans-8-methyl-N-vanilyl-6-nonenamide), the pungent substance of 'hot' chilli peppers (*Capsicum* species) [16]. TRPV1 activation produces burning pain mediated by cation influx. This leads to nociceptor excitation and neuropeptide release followed by receptor desensitisation, nerve degeneration and neuropetide depletion, which causes a longer-lasting analgesia [17]. Thus, capsaicin is clinically used for the management of neuropatic pain syndromes and arthritis, despite its undesirable initial irritant effect. Several natural products mimic capsaicin action, such as the ultrapotent analogue resiniferatoxin (isolated from the cactus-like plant *Euphorbia resinifera*) [17], the unsaturated 1,4-dialdehydes polygodial, isovelleral [18] and gingerols (isolated from *Ginger* species) [19]. However, the development of drugs that selectively interact with TRPV1 and do not display pungent properties would be of potential therapeutic value. Indeed, some substances such as the capsaicin analogues olvanil and SDZ 249-665 [20], the *Ricinus communis* isolated compound ricinoleic acid [21] and the triphenyl phenol scutigeral [22] show analgesic action but do not produce initial irritation. This is also true for the heat-activated channel TRPV1. TRPM8, an ionotropic receptor sensitive to cold and menthol, was recently cloned [23,24]. This receptor may be an important target for drugs able to reduce the cold-allodynia observed in patients with chronic pain.

The activation of nociceptors in the periphery produces messages which converge in the dorsal horn of the spinal cord. This processes the nociceptive information and projects it to the cerebral cortex via a relay in the thalamus, eliciting pain [25]. These central nervous system mechanisms depend on a balance between pro-nociceptive (such as glutamate, P substance) and anti-nociceptive (such as opioids, cannabinoids) transmitter release and action.

*Papaver somniferum* Papaveraceae (opium poppy) has been used as an analgesic since 3000 BC [26]. The opium latex contains many alkaloids, including codeine, thebaine, papaverine and morphine - its main analgesic constituent [27]. Although morphine has found large clinical use, its analgesic mechanism was first elucidated only with the discovery of both endogenous peptide opioids (such as enkephalins, dynorphins, endorphins and nociceptin) and the opioid receptors, $\mu$, $\delta$, $\kappa$

and OLR-1 [28]. Opioid receptors complex with pertussis toxin sensitive-G$\alpha$i protein which produces hyperpolarisation of sensory neurones and inhibition of pro-nociceptive transmitter release mediated by a reduction in intracellular cAMP levels, blockade of the $Ca^{2+}$ channels and activation of the $K^+$ ones [28]. Along with their analgesic effects, opioids also produce some well-known undesirable side effects such as constipation, urine retention, euphorogenic properties, appetite suppression, hypothermia, bradycardia and tolerance [1,2]. Nevertheless, morphine and its analogues still continue to be some of the most used drugs in clinical practice due to their efficiency for several painful conditions.

Advances in the understanding of endogenous control of pain were obtained with cannabinoids. Throughout history, *Cannabis sativa* L. has been used as a natural therapeutic herb as well as an analgesic. The *Cannabis* plant contains a complex mixture of substances that include at least 60 different cannabinoids, including $\Delta^9$-tetrahydrocannabinol (THC) [29]. Pharmacological studies have demonstrated that THC and other active cannabinoids act at specific receptors called CB1 and CB2 [30]. The cannabinoid receptors are expressed in both the peripheral and central nervous system and are endogenously activated by polyunsaturated fatty acids, such as anandamide and palmitoylethanolamine [31]. It is believed that the cannabinoids are capable of controlling neuronal excitability. Cannabinoids exert anti-nociceptive effects by central and peripheral action that may be assessed in several experimental models, especially in chronic pain [31]. This is an important point, since pharmacotherapy for chronic pain has been unsuccessful either due to limited efficacy or the production of undesirable side-effects [32]. When orally administered, THC has acted as an analgesic in patients suffering from advanced cancer, familiar Mediterranean fever and migraine [33,4]. There is a wide range of possibilities for the use of cannabinoids in the treatment of pain, but until adequate and well-controlled double-blind clinical trials can be conducted, the therapeutic exploitation of cannabinoids will remain no more than a future possibility.

In view of such multiplicity of mechanisms known to modulate pain transmission, reports on many compounds that can directly or indirectly modulate pain transmission are not surprising. However, few of them reach sufficient selectivity of action and consequently develop clinical interest.

## New Developments Based on Structure-Activity Relationships

Salicin, a glycoside obtained from the willow bark, particularly *Salix fragilis* and *Salix purpurea*, was the lead compound for the synthesis of aspirin (acetylsalicylic acid), based on considerations of structure-properties and structure-activity relationships. Great effort has since been put into obtaining analogues for the natural analgesic products: $\Delta^9$-tetrahydrocannabinol, morphine and capsaicin. The objective is to obtain compounds which demonstrate potent analgesic activity but no undesirable effects. The present investigation was oriented, as in the case of aspirin, towards studies on structure-activity relationships looking into the fundamental structural aspects responsible for the activity.

*Cannabinoids*

Cannabinoids are natural bi- and tricyclic products (approximately 60) isolated from *Cannabis sativa* which have a structure related to $\Delta^9$-tetrahydrocannabinol (THC).

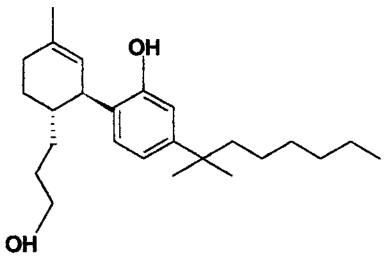
Several studies on the structure-activity relationships (SAR) of cannabinoids have been recently published [34-37]. Three important features have been determined for classical and nonclassical cannabinoids: a side-chain of three to seven carbon atoms in the 3-position of the phenol, a phenolic hydroxyl, and a suitable conformation or substituents in the C 9 position. However, not all these features are necessarily required to produce analgesic effects.

Recently, Keimowitz et al [38], aware that the cannabinoid side chain is a key pharmacophore, studied a series of 36 side-chain substituted $\Delta^8$-THCs with a wide range of pharmacological potency using computational molecular modelling and QSAR analysis. They showed that for optimum affinity and potency, the side chain must have conformational freedom that allows its terminus to fold back and come into proximity with the phenolic ring. *In vivo* THC is oxidized to the more potent 11-OH-$\Delta^9$-THC. Modifications of this structure yield 9-nor-9β-OH-hexahydrocannobinol (HHC) that has exhibited enhanced analgesic activity.

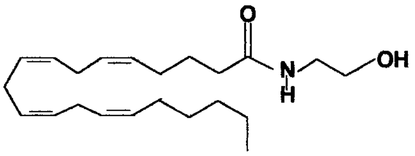
Anandamide is proposed as an endogenous cannabinoid that has been shown to mimic the effects of tetrahydrocannabinol in both *in vivo* and *in vitro* systems. Thus, THC and anandamide have common pharmacological properties despite their different structures. The former is a rigid compound whereas the latter is flexible.

Khanolkar and Makriyannis [39] have studied the SAR of anandamide for the CB1 cannabinoid receptor indicating the structural requirements of the two major molecular fragments: the polar ethanolamido head group and the hydrophobic arachidonyl chain. A number of researchers have struggled to develop a pharmacophore that can fit the cannabinoids related to THC and anandamide. By employing a combination of Dreiding molecular models and the PC Model program, Huffman et al [40] have developed a model where the amide carbonyl of anandamide is superimposed with the phenolic hydroxyl of $\Delta^9$-THC and the terminal carbons of anandamide are aligned with the alkyl side chain of $\Delta^9$-THC. The amide nitrogen of anandamide is aligned with C-2 of THC and the N-substituent of anandamide is considered to correspond to a C-2 substituent on the cannabinoid nucleus. Considering this model, Huffman et al synthesised a pyridone cannabinoid that has moderately high affinity for the $CB_2$ receptor (ki < 60 nM).

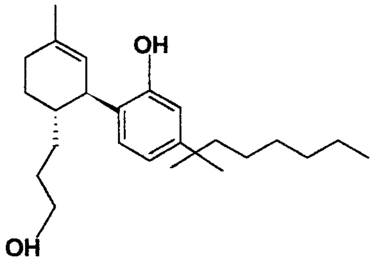
From studies on the comparative molecular field analysis of correlation between tetrahydrocannabinol and anandamide analogues, Thomas et al [41-42] suggested that the oxygen of the phenolic hydroxyl and the pyran oxygen of tetrahydrocannabinol are aligned with the hydroxyl group and carbonyl oxygen of anandamide [41]. Recently, considering the model proposed by Thomas, Martin et al have reasoned that if the hydroxyl groups of both compounds represent a common pharmacophore, then similar substitution on both tetrahydrocannabinol and anandamide should produce similar changes in pharmacological potency and receptor affinity. Thus, these authors prepared 1-fluoro-tetrahydrocannabinol and the derivate of anandamide with the substitution of the hydroxyl group for fluorine. While the fluorine analogue of anandamide increased the $CB_1$ cannabionoid binding affinity approximately 10-fold, the fluorine derivate of tetrahydrocannabinol revealed low affinity for the $CB_1$ cannabinoid receptor and was considerably less potent than tetrahydrocannabinol. These results suggest that these two oxygen moieties do not overlap. Additionally, these analogues have a higher affinity for $CB_2$ than for $CB_1$ cannabinoid receptors showing a fundamental difference in the binding properties of these two receptor subtypes [42].



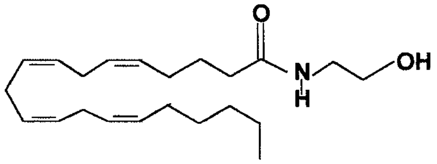

Δ$^9$-THC
CLASSICAL CANNABIONOID



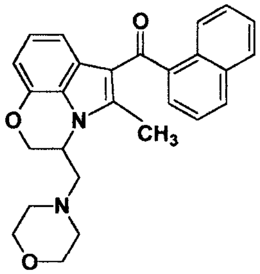

HHC
NON-CLASSICAL CANNABIONOID



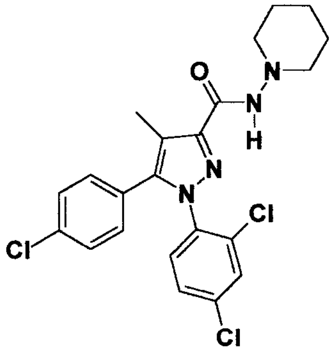

CP-55,940
BICYCLIC CANNABINOIDS




ANANDAMIDE
ENDOCANNABINOIDS




AMINOALKYLINDOLES




DIARYLPYRAZOLES

Fig. 1: The six groups of cannabinoid receptors ligands

However, studies on structure-activity relationships have revealed that, in addition to classical cannabinoids, other structurally different compounds can also be CB receptor ligands. Thus, the term "cannabinoids" is now applied to any compound with good affinity for cannabinoid receptors.

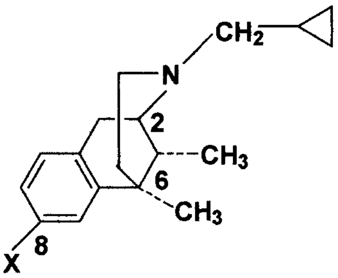
The cannabinoid ligand can be classified according to Ooms et al [43] into six groups: i) classical, ii) nonclassical, iii) bicyclic, iv) aminoalkylindoles, v) endocannabinoid analogues, vi) diarylpyrazoles. Ooms et al [43] studied the pharmacophore of 3-alkyl-5-arylimidazolidinediones as a new $CB_1$ cannabinoid receptor. Thomas et al [44] have studied SAR data on a series of 1,5-diphenylpyrazoles proposing a pharmacophoric alignment of these compounds with THC (Fig.1).

It is clear that, as Barth and Rinaldi-Carmona pointed out in their review on the development of the cannabinoid antagonist, [45] that the millennial therapeutic use of *C. sativa* has led modern medical science to the research of new analgesic compounds based on cannabinoid antagonists.

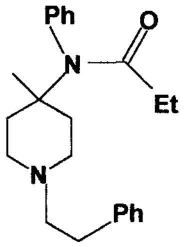
*Opioids*

The demand for a safe, non-addictive, orally active and side-effect-free compound based on the morphine structure has led to several studies on structure-activity relationships (SAR) that have permitted the definition of the pharmacophore group, with regard to the structural requirements necessary to yield analgesic activity. Thus, it has been established that the aromatic ring and the basic nitrogen are essential for activity. Among recently studied morphine analogues, derivatives of bupremorphine, cyclazosine, and fentanyl merit further consideration. HS-595, a new dehydroderivative of bupremorphine (18,19-dehydrobupremorphine) has higher affinity, selectivity and potency than its parent compound [46-47]. It is important to remember that the structure of bupremorphine is more rigid than that of morphine and that the elimination of some side-effects is attributed to this aspect. This makes it a safe drug since it has very little effect on respiration. On the other hand, bupremorphine is more lipophilic and therefore enters the brain very easily.

The 3-carboxamido analogue of cyclazosine (a benzomorphan without the C and D rings of morphine) where the –OH group in position 8 of the aromatic ring was substituted by a carboxamido group

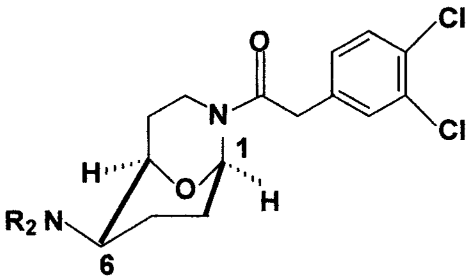



1: X = OH (CYCLAZOCINE)
2: X = $CONH_2$ (3-CARBOXAMIDO ANALOGUE)




6

4-METHYL FENTANYL




$NR_2 = NHCH_2C_2H_5$
AMINO-SUBSTITUTED MORPHAN ANALOGUE

Fig. 2 Some analogues of morphine

($CONH_2$) exhibited a high affinity for opioid receptors (Ki = 0.41 and 0.55 nM for μ and κ opioid receptors, respectively). These values are similar to those for cyclazone. A high enantiopreference (2R, 6R, 11R) for binding was also observed [48]. It is interesting to note that this fact has been neither predicted nor rationalized by current knowledge of SAR for opioid interactive agents.

Other recently studied compounds are the 3-carboxamido analogues of morphine and nactrexone [49].

One important analogue of morphine is fentanyl, a clinically used narcotic that is about 100 times more potent than morphine. Several analogues have been synthesised such as sufentanil, alfentanil, lofentanil and, even more interestingly, structurally related compounds with different activities such as astemizole (antihistaminic), droperidol (tranquillizing), loperamide (antidiarrheal) and lorcainide (antiarrhythmic) [50].

It has been proven that the central analgesic activity in the anilidopiperidines is influenced by steric requirements of the group in position 4 of the piperidine ring since carbomethoxy, methoxymethyl, hydroxymethyl and other groups significantly increase potency compared with fetanyl. Following this finding, Micovic et al [50] synthesized 4-methyl fentanyl. Preliminary pharmacological results indicated a highly potent narcotic analgesic effect, about four times higher than that of fentanyl. Höfner et al [51] have studied some amino-substituted morphan analogues. The morphan ring system represents a substructure of the morphine. Strong analgesia as well as opioid receptor binding are observed with suitable substituted morphan derivatives [52]. In order to find novel ligands for opioid receptors, in particular for κ-receptors, Höfner et al [51] set out to combine pharmacophoric elements for κ-receptors such as the arylacetamide and amine with the morphan skeleton. The most active compound was obtained with a μ-receptor affinity and Ki = 14 nM.

*Vallinoids*

Capsaicin is a pungent ingredient of the *Capsicum* species. Its biological effect is biphasic; the first phase is the excitation of the primary afferents and the second consists of desensitisation or inactivation of neurons [53]. Walpole et al [54] have analysed the structural requirements for capsaicin-like activity in three regions of the molecule: the aromatic ring, the amide bond and the hydrophobic side-chain. These three

regions were the basis for the synthesis of analogues in the search for more potent compounds. However, the potency of the analogues could not be significantly increased, and no better lead emerged from these investigations [55]. The discovery of alternative structures with improved potency and/or pharmacological profiles such as resiniferonoids, N-vanilly-N-(3-acyloxy-2-benzylpropyl)-thioureas, N-alkylhomovanillami- des, and N-vanillyl-N-benzylureas shifted the course of the research towards vanilloids.

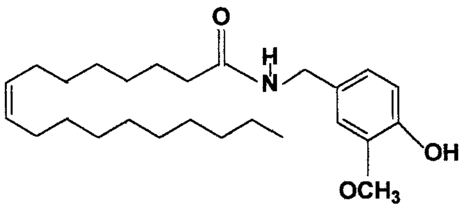
Modification of the amide region and the vanillyl moiety of capsaicin have been systematically carried out. However, the acyl moiety has a high degree of freedom because six torsion angles can be varied in the range of 0-360$^0$C giving a wide range of geometries with hundreds of low energy conformations that are very difficult to submit to a systematic study. In addition, the lipophilic part of the capsaicin receptor has elements that can bind polar groups and structural elements that can pre-organize the conformation of the acyl moiety of capsaicin [55].

There is evidence indicating that the structure-activity requirements for excitation and inhibition are different for capsaicin analogues. Thus, the incorporation of certain long chain fatty acids in capsaicin analogues resulted in desensitising and analgesic activity but also decreased pungency. [56].

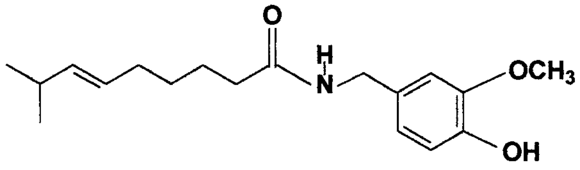
It has been established that the excitatory phase is associated with bronchoconstriction, hypertension, pungent irritation of skin and pain sensation. Among the capsaicin congeners that have been synthesized, olvanil is particularly promising because of its lack of pungency and excellent pharmacological profile which includes analgesia, anti-inflammation and vasorelaxation [57].

Urban [20] described a capsaicin-analogue compound (SDZ 249-665), with antinociceptive and anti-hyperalgesic activity as well as reduced pungency. In addition, it does not cause bronchoconstriction or changes in blood pressure.

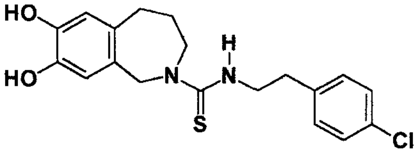
Recently, Oh et al [58] have found that capsazepine, a vanilloid receptor antagonist, inhibited nitric oxide (NO) and iNOS protein synthesis induced by lipopolysaccharide in RAW264.7 macrophages via suppression of iNOS mRNA. Capsaicin is known to have an inhibitory effect on NO production in macrophages. These processes have implications regarding the pathogenesis of inflammatory diseases. According to these authors, capsazipine may be a useful candidate for the development of a drug to treat inflammatory diseases.




OLVANIL




CAPSAICIN




CAPSAZEPINE

Fig.3 Chemical structures of two vanilloid receptor agonists olvanil and capsaicin and of the vanilloid receptor antagonist capsazepine

## NOVEL PLANT COMPOUNDS FOR THE DEVELOPMENT OF ANALGESIC DRUGS

As discussed earlier, natural products have been important in the development of modern analgesics. Thus, the discovery of new analgesics may also be derived from recent work carried out with plant extracts and compounds.

### Glycosides

The ginseng root has been used in traditional folk medicine to relieve certain types of pain, such as toothache, abdominal pain, chest pain or neuralgia [59]. Although seven major species of ginseng (*Panax* species, Araliaceae) are found in nature, most studies have utilised constituents from only three species: *P. ginseng*, *P. quinquefolius* and *P. japonicus* [60]. Ginsenosides, or ginseng saponins, are the main molecules responsible for ginseng action [59]. The ginsenosides have an aglicone with a steroid-like structure and sugar moieties attached. They show properties similar to adrenaline, histamine or opioids [61].

Experimental evidence indicates that ginsenosides acting on the central nervous system reduce chemical stimuli-induced nociception. This effect seems to be produced by voltage-sensitive calcium channels and TRPV1 receptor blockade [62-63]. Experiments have also shown that ginsenosides can inhibit the pain related behaviour produced by peripheral capsaicin injection and that ginsenosides act on both spinal and supraspinal regions. They are not opioid receptors [59]. Opioid drug effects may be partially inhibited by ginseng extracts and ginsenosides. However, this effect was not mediated by direct interaction of opioid receptors [64-65-66]. *In vitro* studies have shown that ginseng and its compounds interact with neurokinin $NK_1$, bradykinin $B_2$ receptors and $GABA_A$ and $_B$ [67-68]. Although the clinical application of ginseng as an analgesic is promising, it relies on additional experimental studies on chronic pain models, investigation of opioid-like undesirable effects and well-conducted clinical trials on painful conditions.

*Kalopanax pictus* is a deciduous tree which grows in Eastern Asia. *Kalopanax* cortex has been used in folk medicine for the treatment of rheumatoidal arthritis. It has been showed that two hederagenin monodesmosides: Kalopanaxsaponins –A and –I present in the extract of

*K. pictus* exhibited significant antinoniceptive effects in addition to inhibition of rheumatoidal arthritis. On Freund's complete adjuvant reagent-induced rheumatoidal arthritis in rats the administration of two hederagenin monodesmoside inhibited edema, agglutination, vascular permeability and trypsin production. These results suggested that the anti-rheumatoidal effects of these compounds are at least in part related to the inhibition of kinin formation by suppression of trypsin inhibitor activity. Thus, hederaginin monodesmodides, or *Kalopanax* cortex, could be considered for the treatment of chronic rheumatoidal arthritis as safe agents from the toxicological point of view [69].

*Dispsacus asper* is a perennial herb distributed across China. Its roots have been used in traditional medicine as an analgesic. Recently, a triterpene glycoside named dispsacus saponin C was isolated. When spinally administered, this compound produces antinociception which appears to be mediated by GABAergic, serotonergic and noradrenergic receptors in the spinal cord [70].

*Alkaloids*

The plant *Siphocampylus verticillatus* (Campanulaceae) is a native species of Brazil. The hydroalcoholic extract from its dried stems and leaves as well as its main active substance - the alkaloid denoted cis-8,10-di-N-propyllobelidiol hydrochloride dihydrate (DPHD) - produce antinociception when assessed in several models of nociception [71-72]. Its antinociceptive effect is antagonised by $\mu$, $\delta$ and $\kappa$ opioid receptor antagonists and by the monoamine-synthesis inhibitor. In fact, the plant extract and DPHD interact with opioid receptor binding and inhibit the re-uptake of the monoamines serotonin, noradrenaline and to a lesser extent, dopamine *in vitro* [73-J.B. Calixto, unpublished results]. In contrast to morphine, DPHD does not seem to produce gastrointestinal side-effects and tolerance [73]. Recently, a second novel piperidine alkaloid hydrochloride with similar antinociceptive action was isolated from *S. verticillatus* [76]. Moreover, *S. verticillatus* extract elicits significant antidepressant-like effect in mice [74]. Interestingly, antidepressants are clinically used as analgesics at doses that produce minor central undesirable effects [75].

*Incarvillea sinensis* has been used in traditional Chinese medicine as a drug to treat rheumatism and relieve pain. Incarvillateine is a monoterpene alkaloid isolated from this plant. When intraperitoneally

injected it produces graded inhibition of both neurogenic and inflammatory pain induced by formalin. The antinociceptive action was partially blocked by naloxone indicating an interaction with the central opioid mechanism. These results suggest that incarvillateine may be a new type of antinociceptive agent with a different mechanism of action to that of morphine [77].

(+) Matrine and (+) allomatrine are two alkaloids present in some *Sophora* species that are used in traditional Chinese medicine. The antinociceptive effect of these alkaloids were examined using the acetic acid-induced abdominal contraction test. It has been shown that the antinociceptive effect of (+) allometrine is mediated through the activation of kappa-opioid receptors, whereas the antinociceptive effect of (+)-matrine was mediated by both mu and kappa-opioid receptors. (-)-sophoridine, the C-6 epimer of (+)-matrine, (+)-sophoranol, (-)-14 β-hydroxymatrine and (+)-matrine N-oxide, which each possess a hydrophilic group and (-)-sophocarpine and (-)-sophoramine, each having a double bond, do not show significant antinociceptive activity [78]. Recently, it has been reported that (+)-matrine and (+)-allomatrine have antinociceptive activity mediated mainly by the activation of kappa-opioid receptors. 1-Acyl-4-dialkylaminopiperidines were synthesised as simple derivatives of matrine and the structure-activity relationship was studied suggesting that the amide group of (+)-matrine is an essential functional group for the antinociceptive potency [79].

Surveys on medicinal plants used among Amazonian rural peasants in the State of Pará (Brazil) pointed to *Psycotria colorata*, traditionally used in the treatment of earache as well as to relieve abdominal pain. The trimmer hodgkinsine is one of the most abundant pyrrolidinoindol alkaloids present in *Psycotria* species extracts. Pharmacological analysis revealed that hodgkinsine produces a dose-dependent naloxone reversible analgesic effect in thermal models of nociception, suggesting that activation of opioid receptors participates in its mode of action. Hodgkinsine exhibits antinociceptive action in the capsaicin model of pain [80]. The same authors suggested that the glutamate NMDA receptors are to be likely involved in its action [80]. More recently, SAR studies have been initiated with the synthesis of three diastereomeric dimmers (chimonanthines). Strong binding affinities for mu-opioid receptors were found for (-) and (+)-chimonanthine monourethanes whereas (-), (+) and (meso)-chimanonthine and hodgkinsine displayed low affinity [81].

Bark of the *Drymis winteri* (Winteraceae) tree is commonly used in Brazilian folk medicine as an anti-inflammatory and as a culinary substitute for pepper. The hydroalcoholic extract of *D. winteri* and its main compound - the sesquiterpene polygodial - possess anti-inflammatory and antinociceptive properties [82-83-84-85]. Polygodial effects seem to be mediated by capsaicin receptor (TRPV1) interaction [see discussion above; Szallasi et al., 18]. Neonate rats treated with capsaicin or polygodial showed antinociception and reduction of neurogenic inflammation [J.B. Calixto, unpublished results]. However, the capsaicin effect is accompanied by TRPV1 expression reduction, contrary to what was observed for the polygodial effect. Polygodial may exert its effects by TRPV1 interaction differently from capsaicin. Drimanial is another polygodial-related sesquiterpene isolated from the bark of *D. winteri* which also possesses antinociceptive effect partially mediated by TRPV1 [86]. Moreover, the antinociceptive effect of drimanial seems to be related to metabotropic glutamate receptor interaction [87].

*Tanacetum parthenium* (Asteraceae), commonly known as feverfew, is a popular herbal remedy used a prophylactic in the treatment of migraine [88]. Studies have revealed that the action of feverfew is probably mediated by the sesquiterpene lactone parthenolide. Indeed, feverfew and parthenolide produce anti-inflammatory and antinociceptive effects in experimental animals [89]. Parthenolide is a potent inhibitor of the transcription factor NF-κB activation, a key regulator of pro-inflammatory protein production, such as cytokines, COX-2 and inducible nitric oxide synthase [90]. However, a clinical study revealed that feverfew did not provide any benefit in the treatment of rheumatoid arthritis [91]. Additional clinical studies must be carried out to explore the feverfew efficacy as an analgesic.

The production of pro-inflammatory substances is an important step during chronic painful inflammatory diseases and its inhibition is a target of novel analgesics. The resin of *Protium kleinii* is used in folk medicine for inflammatory skin conditions. Its effects seem to be mediated by several pentacyclic triterpenes such as amyrin and brein [92]. The topical and systemic administration of the extract of *P. kleinii* and some of its triterpenes has antinociceptive and anti-inflammatory actions, accompanied by reduced neutrophil infiltration and cytokine

production at the site of the injury [J.B. Calixto, unpublished results]. Moreover, oral administration of the pentacyclic triterperpene tormentic acid produces antinociceptive effect in chronic experimental models of arthritic and neurophatic pain [26]. The effect of pentacyclic triterpenes seems to be mediated by inhibition of protein kinases such as protein kinase A and C [93].

Menthol is a cyclic alcohol present in the volatile oil of several species of mint such as *Mentha piperita* and *Mentha aevensis*. It is responsible for the typical minty smell and flavour. (-)-Menthol produced a dose-dependent increase in the pain threshold in the mouse hot-plate and abdominal constriction tests. The antinociceptive effects of (-)-menthol were antagonized by the unselective opioid antagonist naloxone and by the selective kappa-antagonist nor-NBI. Thus, menthol has analgesic properties mediated by selective activation of kappa-opioid receptors.

*Flavonoids*

*Rheedia gardneriana*, a plant used in traditional medicine in Brazil, contains some biflavonoids that exhibit antinocicetive effects. 13-narin-II8-4'-Ome-eridictyol is a biflavonoid which contains a methoxyl group in position 4 of ring-II. It has revealed potent dose-dependent antinociceptive action during writhing and formalin tests. Results have shown that the effect of this biflavonoid against the second phase of the formalin test was not associated with inhibition of paw oedema. Further studies are necessary to elucidate the mechanism of this compound [94].

*Morus nigra*, known as 'amoreira-preta' in Brazil, is widely employed by folk medicine in many parts of the world as an anti-inflammatory and for pathologies associated with dolorous process [95]. It has been demonstrated that morusin, the main prenylflavonoid of this plant, exerts antinociceptive effects on different animal models *in vivo*. This result suggests that the mechanisms involve the participation of the opioid system [96].

## Miscellaneous Compounds

A great number of species from the *Phyllanthus* (Euphorbiaceae) genus are widely used in folk medicine to treat hepatitis and other kidney disturbances [97]. In Brazil, Phyllanthus species are widely used to treat

painful conditions of urinary lithiasis. In fact, previous studies carried out by our group have demonstrated that the extracts obtained from the leaves, stems and roots of several species of plants exhibit pronounced antinociception [97]. There is vast evidence suggesting that many classes of compounds present in most plants of the *Phyllanthus* genus are involved in the antinociceptive action reported for such plants, such as gallic acid ethyl ester, furosin, geraniin, quercetin, rutin and stigmasterol. We have shown that the antinociception of gallic acid ethyl ester is caused by $K^+$ channel modulations, which can produce nociceptive fibre hyperpolarisation and release of pro-nociceptive neurotransmitters [71]. An important QSAR study was carried out focusing on the analgesic activity of gallic acid derivatives. The developed QSAR model has substantial predictive power for the design of novel gallic acid derivatives, having improved potency [98]. The importance of the data demonstrating that quercetin (but not rutin or geraniin) present in the *Phyllanthus* species is capable of inhibiting [$^3$H]-glutamate and GTP analogue [$^3$H] GMPPNP binding at glutamate receptors in rat brain membranes [99] should also be noted. As clinical trials have demonstrated that some of the extracts of *Phyllanthus* were well tolerated in humans with no evidence of side effects [97-2], it is expected that these plants should be of clinical interest to promote the development of well-controlled and standardised phytomedicinals for the management of painful disorders.

## REFERENCES


1. Calixto, J.B.; Beirith, A.; Ferreira, J.; Santos, A.R.; Filho, V.C.; Yunes, R.A.; *Phytother. Res.*; **2000**, 14, 401-418.
2. Calixto, J.B.; Scheidt, C.; Otuki, M.; Santos, A.R.; *Exp. Opin. Emerging Drugs*, **2001**, 6, 1-18.
3. IASP Task Force on Taxonomy. In: Classification of Chronic Pain, Merskey, H.; Bogduk, N., Ed; *IASP Press: Seattle*, **1994**; Second edition; 209-214.
4. Russo, C.M.; Brose, W.G.; *Ann. Rev. Med.*, **1998**, 49, 123-133.
5. Besson, J.M.; *Lancet*, **1999**, 353, 1610-1615.
6. Julius, D.; Basbaum, A.I.; *Nature*, **2001**, 413, 203-210.
7. Vane, J.R.;Botting, R.M.; *Inflamm. Res.*, **1995**, 44, 1-10.
8. Vane, J.R.; *Nature*, **1971**, 231, 232-235.
9. Ferreira, S.H.; *Nature*, **1972**, 240, 200-203.
10. O'Banion, M.K.; *Crit. Rev. Neurobiol.*, **1999**, 13, 45-82.
11. Chandrasekharan, N.V.; Dai, H.; Roos, K.L.; Evanson, N.K.; Tomsik, J.; Elton, T.S.; Simmons, D.L. *Proc. Natl. Acad. Sci. USA*, **2002**, 99, 13926-13931.
12. Bley, K.R.; Hunter, J.C,; Eglen, R.M.; Smith, J.A.; *Trends Pharmacol. Sci.*, **1998**, 19, 141-147.

13. Khasar, S.G.; Gold, M.S.; Levine, J.D.; *Neurosci. Lett.*, **1998**, 256, 17-20.
14. De Petrocellis, L.; Harrison, S.; Bisogno, T.; Tognetto, M.; Brandi, I.; Smith, G.D.; Creminon, C.; Davis, J.B.; Geppetti, P.; Di Marzo, V.; *J. Neurochem.*, **2001**, 77, 1660-1663.
15. Caterina, M.J.; Julius, D.; *Annu. Rev. Neurosci.*, **2001**, 24, 487-517.
16. Caterina, M.J.; Schumacher, M.A.; Tominaga, M.; Rosen, T.A.; Levine, J.D.; Julius, D; *Nature*, **1997**, 389, 816-824.
17. Szallasi, A.; Blumberg, P.M.; *Neuroscience*, **1989**, 30, 515-220.
18. Szallasi, A.; Jonassohn, M.; Acs, G.; Biro, T.; Acs, P.; Blumberg, P.M.; Sterner, O.; *Br. J. Pharmacol.*, **1996**, 119, 283-290.
19. Dedov, V.N.; Tran, V.H.; Duke, C.C.; Connor, M.; Christie, M.J.; Mandadi, S.; Roufogalis, B.D.; *Br. J. Pharmacol.*, **2002**, 137, 793-798.
20. Urban, L.; Campbell, E.A.; Panesar, M.; Patel, S.; Chaudhry, N.; Kane, S.; Buchheit, K.; Sandells, B.; James, I.F.; *Pain*, **2000**, 89, 65-74.
21. Vieira, C.; Evangelista, S.; Cirillo, R.; Terracciano, R.; Lippi, A.; Maggi, C.A.; Manzini, S.; *Eur. J. Pharmacol.*, **2000**, 407, 109-116.
22. Szallasi, A.; Biro, T.; Szabo, T.; Modarres, S.; Petersen, M.; Klusch, A.; Blumberg, P.M.; Krause, J.E.; Sterner, O.; *Br. J. Pharmacol.*, **1999**, 126, 1351-1358.
23. McKemy, D.D.; Neuhausser, W.M.; Julius, D.; *Nature*, **2002**, 416, 52-58.
24. Peier, A.M.; Moqrich, A.; Hergarden, A.C.; Reeve, A.J.; Andersson, D.A.; Story, G.M.; Earley, T.J.; Dragoni, I.; McIntyre, P.; Bevan, S.; Patapoutian, A.; *Cell*, **2002**, 108, 705-715.
25. Woolf, C.J.; Salter, M.W.; *Science*, **2000**, 288, 1765-1768.
26. Bortalanza, L.B.; Ferreira, J.; Hess, S.C.; Delle Monache, F.; Yunes, R.A.; Calixto, J.B.; *Eur. J. Pharmacol.*, **2002**, 453, 203-208
27. Trease, G.E.; Evans, W.C., *Pharmacognosy. Billière Tindall*, **1978**.
28. Dhawan, B.N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P.B.; Portoghese, P.S.; Hamon, M.; *Pharmacol. Rev.*, **1996**, 48, 567-591.
29. Gaoni, Y.; Mechoulam, R.; *J. Am. Chem. Soc.*, **1964**, 86, 1646-1647.
30. Matsuda, L.A.; Lolait, S.J.; Brownstein, M.J.; Young, A.C.; Bonner, T.I.; *Nature*, **1990**, 346, 561-564.
31. Hirst, R.A.; Lambert, D.G.; Notcutt, W.G.; Br. J. *Anaest.*, **1998**, 81, 77-84.
32. Woolf, C.J.; Mannion, R.J.; *Lancet*, **1999**, 353, 1959-1964.
33. Holdcroft, A.; Smith, M.; Jacklin, A.; Hodgson, H.; Smith, B.; Newton, M.; Evans, F.; *Anaesthesia*, **1997**, 52, 483-488.
34. Seltzman H.H., *Curr. Med. Chem.*, **1999**, 6, 685-704.
35. Pertwee R.G., *Curr. Med. Chem.*, **1999**, 6, 635.
36. Huffman J.W., *Curr. Med. Chem.*, **1999**, 6, 705.
37. Di Marzo V., De Petrocellis L., Melck D., Martin B.R., *Curr. Med. Chem.*, **1999**, 6, 721.
38. Keimowitz A.R., Martin B.R., Razdan R.K., Crocker P.J., Mascarella S.W., Thomas B.F., *J. Med. Chem.*, **2000**, 42, 59-70.
39. Khanolkar A.D., Makriyannis A., *Life Sci.*, **1999**, 65, 607-616.
40. Huffman J.W., Lu J., Hynd G., Wiley J.L., Martin B.R., *Bioorg.Med. Chem.*, **2001**, 9, 2863-2870.
41. Thomas B.R., Adams I.B., Mascarella S.W., Martin B.R., Razdan R.K., *J. Med. Chem.* **1996**, 39, 471-479.

42. Martin B.R., Jefferson R.G., Winckler R., Wiley J.L., Thomas B.F., Crocker P.J., Williams W., Razdan R.K., *Eur. J. Pharmacol.*, **2000**, 435, 35-42
43. Ooms F., Wouters J., Oscari O., Happaerts T., Bouchard G., Garrup P., Tese B., Lembert D.M., *J. Med. Chem.*, **2002**, 45, 1748-56.
44. Thomas B.F., Gilliam A.F., Burch D.F., Roche M.J., Seltzman H.H., J. *Pharmacol. Exp. Ther.*, **1998**, 285, 285-292.
45. Carth F., Rinaldi-Carmona M., *Curr. Med. Chem.*, **1999**, 6, 745-755.
46. Lattanzi R., Negri L., Giannini E., Schmidhammer H., Schutz J., Improta G., *Br. J. Pharmacol.*, **2001**, 134, 441-447.
47. Lattanzi R., Negri L., Schmidhammer H., Giannini E., *Life Sci.*, **2002**, 70, 2177-85.
48. Wentland M.P., Lou R., Ye Y. Cohen D.J., Richardson G.P., Bidlack J.M., *Bioorg.Med. Chem. Lett.*, **2001**, 11, 623-626.
49. Wentland M.P., Lou R., Dehnhardt C.M., Duan W., Cohen D.J., Bidlack J.M., *Bioorg Med Chem Lett.*, **2001**, 11,1717-21.
50. Micovic I.V., Ivanovic M.D., Vuckovic S.M., Prostran M.S., Dosen Micovic L., Kiricojevic V.D., *Bioorg. Med. Chem. Lett.*, **2000**, 10, 2011-14.
51. Höfner G., Streicher B., Münsch B., Arch. *Pharm. Med. Chem.*, **2001**, 334, 284-290.
52. Thomas K.M., Fix S.E., Burgess J.P., Cooper J.B., Mascarella S.W., Cantreall B.E., Zimmermamm M.D., Carroll F.I., *Tetrahedron Lett.*, **1999**, 40, 403-406.
53. Dray A., Bettany J., Forster P., *Br. J. Pharmacol*, **1999**, 101, 727-33.
54. Walpole C.S., Wrigglesworth R., Bevan S., Campbell E.A., Dray A., James I.F., Masdin K.J., Perkins M.N., Winter J., J. Med. Chem., **1993a**, 36, 2362-72, *J Med Chem.*, **1993b**, 36, 2373-80, *J Med Chem.*, **1993c**, 36, 2362-72
55. Appendino G., Minassi A., Morello A.S., De Petrocellis L., Di Marzo V., *J Med Chem.*, **2002**, 45, 3739-45.
56. Szolcsanyi J., Jancso-Gabor A., *Arzneimittelforschung*, **1975**, 25, 1877-81.
57. Beltramo M. and Piomelli D., *Eur J Pharmacol.*, **1999**, 364, 75-8
58. Oh G.S., Pae H.O., Seo W.G., Kim N.Y., Pyun K.H., Kim I.K., Shin M., Chung H.T., *Int. Immunopharmacol*, **2001**, 1, 777-84
59. Nah, J.J.; Hahn, J.H.; Chung, S.; Choi, S.; Kim, Y.I.; Nah, S.Y.; *Neuropharmacology*, **2000**, 39, 2180-2184.
60. Attele, A,; Wu, J.A.; Yuan, C.S.; *Biochem. Pharmacol.*, **1999**, 58, 1685-1693.
61. Kaku, T.; Miaya, T.; Uruno, T.; Sako, I.; Kinoshita, A. *Drug Res.*, **1975**, 25, 539-547.
62. Hahn, J.; Nah, S.Y.; Nah, J.J.; Uhm, D.Y.; Chung, S.; Neurosci. Lett., **2000**, 287, 45-48.
63. Rhim, H.; Kim, H.; Lee, D.Y.; Oh, T.H.; Nah, S.Y.; *Eur. J. Pharmacol.*, **2002**, 436, 151-158.
64. Nah, S.Y.; McCleskey, E.W.; *J. Ethnopharmacol.*, **1994**, 42, 45-51.
65. Nemmani, K.V.; Ramarao, P.; *Life Sci.*, **2002**, 70, 1727-1740.
66. Ramarao, P.; Bhargava, H.N.; *Gen. Pharmacol.*, **1990**, 21, 877-880.
67. Kimura, T.; Saunders, P.A.; Kim, H.S.; Rheu, H.M.; Oh, K.W.; Ho, I.K.; *Gen. Pharmacol.*, **1994**, 25, 193-199.
68. Sampson, J.H.; Phillipson, J.D.; Bowery, N.G.; O'neill, M.J.; Houston, J.G.; Lewis, J.A.; *Phytother. Res.*, 2000, 14, 24-29.

69. Choi, J.; Huh, K., Kim, S.H.; Park, H.J.; Han, Y.N. *J. Ethnopharmacol*, **2002**, 79, 199-204.
70. Suh, H.W.; Song, D.K.; Huh, S.O.; Son, K.H.; Kim, I.H. *J. Ethnopharmacol*, **2000**, 71, 211-218.
71. Santos, A.R.S.; Miguel, O.G.; Yunes, R.A.; Calixto, J.B.; *J. Pharmacol. Exp. Ther.*, **1999**, 289, 417-426.
72. Trentin, A.P.; Santos, A.R.S.; Miguel, O.G.; Pizzolatti, M.G.; Yunes, R.A.; Calixto, J.B.; *J. Pharm. Pharmacol.*, **1997**, 49, 576-572.
73. Santos, A.R.S.; De Campos, R.O.; Miguel, O.G.; Cechinel Filho, V.; Yunes, R.A.; Calixto, J.B.; *Eur. J. Pharmacol.*, **1999**, 379, 7-17.
74. Rodrigues, A.L.; da Silva, G.L.; Mateussi, A.S.; Fernandes, E.S.; Miguel, O.G.; Yunes, R.A.; Calixto, J.B.; Santos, A.R.;. *Life Sci.*, 2002, 70, 1347-1358.
75. Watson, C.P.; *Clin. J. Pain*, **2000**, 16, S49-S55.
76. Miguel, O.G.; Santos, A.R.S.; Calixto, J.B.; Monache, F.D.; Yunes, R.A. *Z. Naturforsch*, **2002**, 57, 81-84.
77. Nakamura, M.; Chi, Y.M.; Yan, W.M.; Yoshizawa, T.; Irino, N.; Hashimoto, F.; Kinjo, J.; Nohara, T.; Sakurada, S. *J. Nat. Prod.*, **1999**, 62, 1293-1294.
78. Xiao, P.; Kubo, H.; Ohsawa, M.; Higashiyama, K.; Nagase, H.; Yan, Y.N.; Li, J.S.; Kamei, J.; Ohmiya, S. *Planta Med.*, **1999**, 65, 320-233.
79. Kobashi, S.; Kubo, H.; Yamauchi, T.; Higashiyama, K. *Biol. Pharm. Bull.*, **2002**, 25, 1030-1034
80. Amador, T.A.; Verotta, L.; Nunes, D.S., Elisabetsky, E. *Planta Med.*, **2000**, 66, 1-6
81. Verotta, L.; Orsini, F.; Sbacchi, M.; Scheildler, M.A.; Amador, T.A.; Elisabetsky, E. *Biorg. Med. Chem*, **2002**, 10, 2133-2142
82. da Cunha, F.M.; Frode, T.S.; Mendes, G.L.; Malheiros, A.; Cechinel Filho, V.; Yunes, R.A.; Calixto, J.B.; *Life Sci.*, **2001**, 70, 159-169.
83. Mendes, G.L.; Santos, A.R.; Malheiros, A.; Filho, V.C.; Yunes, R.A.; Calixto, J.B.; .*J. Pharmacol. Exp. Ther.*, **2000**, 292, 164-172.
84. Mendes, G.L.; Santos, A.R.; Campos, M.M.; Tratsk, K.S,; Yunes, R.A.; Cechinel Filho, V.; Calixto, J.B. *Life Sci.*, **1998**, 63, 369-381.
85. Tratsk, K.S.; Campos, M.M.; Vaz, Z.R.; Filho, V.C.; Schlemper, V.; Yunes, R.A.; Calixto, J.B.; *Inflamm. Res.*, **1997**, 46, 509-514.
86. Malheiros, A.; Filho, V.C.; Schmitt, C.B.; Santos, A.R.S.; Scheidt, C.; Calixto, J.B.; Monache, F.D.; Yunes, R.A.; *Phytochemistry*, **2001**, 57, 103-107.
87. Scheidt, C.; Santos, A.R.; Ferreira, J.; Malheiros, A.; Cechinel-Filho, V.; Yunes, R.A.; Calixto, J.B.; *Neuropharmacology*, **2002**, 43, 340-347.
88. Ernst, E.; Pittler, M.H.; Public Health. *Nutr.*, **2000**, 3, 500-514.
89. Jain, N.K.; Kulkarni, S.K.; *J. Ethnopharmacol.*, **1999**, 68, 251-259.
90. Hehner, S.P.; Hofmann, T.G.; Droge, W.; Schmitz, M.L.; *J. Immunol.*, **1999**, 163, 5617-5623.
91. Pattrick, M.; Heptinstall, S.; Doherty, M.; *Ann. Rheum. Dis.*, **1989**, 48, 517-549.
92. Otuki, M.F.; Lima, F.V.; Malheiros, A.; Cechinel-Filho, V.; Delle Monache, F.; Yunes, R.A.; Calixto, J.B.; *Life Sci.*, **2001**, 69, 2225-2236.

93. Hasmeda, M.; Kweifio-Okai, G.; Macrides, T.; Polya, G.M.; *Planta Med.*, **1999**, 65, 14-18.
94. Graves, G. **1996**, *Medicinal Plants*, Bracken Books, London
95. Cechinel Filho, V.; Silva, K.L.; de Souza, M.M.; Oliveira, A.E.; Yunes, R.A.; Guimarães, C.L.; Verdi, L.G.; Sinionatto, E.L.; Monache, F.D. *Z. Naturforch.*, **2000**, 55, 820-823
96. de Souza, M.M.; Bittar, M.; Cechinel Filho, V.; Yunes, R.A.; Messana, I.; Monache, F.D.; Ferrari, F. *Z. Naturforch.*, **2000,** 55, 256260
97. Calixto, J.B.; Santos, A.R.; Filho, V.C.; Yunes, R.A.; Med. Res. Rev.; **1998,** 18, 225-258.xc
98. Martini, L.H.; Souza, C.R.; Marques, P.B.; Calixto, J.B.; Yunes, R.A.; Souza, D.O.; Neurochem. Res., **2000**, 25, 211-215.
99. Krogh, R.; Yunes, R.A.; Adricopulo, A.D. *Il Fármaco*, **2000**, 55, 730-735

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# ACID-TREATMENT OF ARYLPROPANOID AND ARYLETHANOID DERIVATIVES – ACCESS TO NATURAL AND SYNTHETIC BIOLOGICALLY ACTIVE POLYPHENOLS

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**ABSTRACT**: The acid-treatment of arylpropanoid derivatives may lead to numerous polyphenols presenting a wide variety of biological properties such as anti-inflammatory, anticancer and antiviral activities and may constitute an alternative route to neolignans to the classical oxidative dimerisation of arylpropenoid compounds. Firstly, I will present the acidic dimerisation of arylethanals, aryllactic acids and 2-aminoarylpropanoic acids. The access to 2-arylnaphthalenes, epoxydibenzocyclooctenes or substituted pavinans may be easily controlled by the acidic conditions. Some natural polyphenols may be obtained. Then, the facile conversion of arylacetones into substituted 1,3-dimethyl-2-arylnaphthalenes using boron tribromide as dimerisation and demethylation agent will be examined. Next, acid-treatment of arylpyruvic acids will be discussed. Depending on the acidic conditions, arylpyruvic acids may simply be isomerised, cyclised into benzofurans or benzocoumarins or dimerised into vitamin C analogues. Lastly, the easy conversion of methoxylated stilbenes into 2-arylbenzofurans presenting the structural feature of moracins or norlignans will be presented. Finally, the biological relevance of the present molecules will be also deeply discussed.

## INTRODUCTION

The treatment of saturated enolisable aldehydes and ketones with acids is known to result in aldol condensation [1]. In the case of arylethanoid derivatives, this treatment results concomitantly in aldol condensation followed by cyclisation and aromatisation. Furthermore, when the acid used is known to promote the demethylation of methoxyphenyl derivatives into phenols, this reaction appears to lead to a great structural diversity of biologically active natural and synthetic polyphenols. In this review, I will focus on the reaction of arylpropanoid and arylethanoid derivatives with acids, mainly boron tribromide but also simple protic acids. The former allows, in a one-pot procedure, aldol condensation, cyclisation, aromatisation and demethylation. The latter may be used

alternatively in order to access otherwise inaccessible structures using boron tribromide and followed by a treatment with $BBr_3$ to give the target polyphenols.

## ARYLETHANALS

Acid treatment of arylethanals may lead to 2-phenylnaphthalenes or 1,2,9,10-tetrahydro-1,9-epoxydibenzo[a,e]cyclooctenes (Kagan's ether). 2-Phenylnaphthalenes were obtained by a C-condensation of the enolated form on the keto form. The resulting aldol condensation product underwent an intramolecular reaction to give, after rearomatisation, the 2-phenylnaphthalenes, Fig. (**1**).

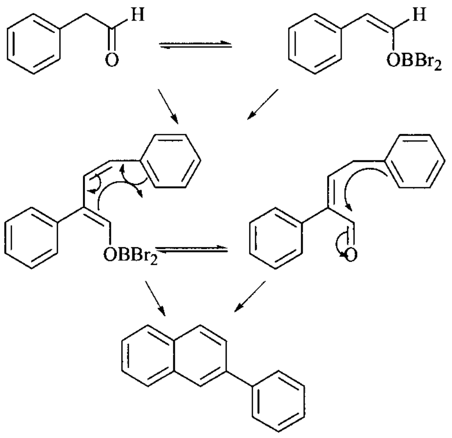



**Fig. (1).** Mechanism of formation of 2-phenylnaphthalenes (example is given with boron tribromide as Lewis acid)

Kagan's ethers were the result of a O-condensation of the ketal derivatives on the keto form. The resulting hemiacetal cyclised to give a benzisopyran, which cyclised by an intramolecular Friedel-Crafts reaction to give Kagan's ethers, Fig. (**2**). The balance between the condensation at O- or C-position depends on the nature of the acid and the substitution on the aryl ring. For example, trimethylsilyliodide [2] and boron tribromide [3] react smoothly with arylethanals to give respectively iodosilylethers and bromoboronylethers.

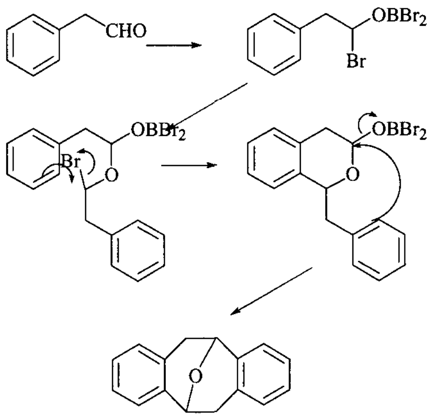



**Fig. (2).** Mechanim of formation of Kagan's ether (example is given with boron tribromide as Lewis acid)

While iodosilylethers are transformed into Kagan's ethers whatever the ring substitution (phenylethanal [2], or 3,4-dimethoxyphenylethanal [4]), bromoboronylethers are only converted into Kagan's ethers when the aryl ring is substituted by electron donating susbtituants [3]. Using these reagents, high yields in Kagan's ether may be obtained and the cyclisations are regioselective. The fact that 2-phenylnaphthalene can be isolated in modest yield from the reaction of phenylethanal with boron tribromide, indicated that $BBr_3$ reaction conditions are not favourable for the double Friedel-Crafts cyclisation and do not occur when the aromatic ring is not electronically rich enough. Kagan's pioneer work [5,6] used strong Brönsted acid, i.e., fluorosulfonic acid, and other protic acids (HCl or $H_2SO_4$) [7,3] have been used in order to promote the aldol condensation of arylethanals. The yields in isolated products are generally poor, again depending on the aromatic substitution. Nevertheless, concentrated HCl in dioxane gives satisfactory yields in O- and/or C-condensation products when dioxane is freshly distilled on sodium and benzophenone (in order to avoid free radicals). Here again, arylethanals substituted by electron-donating groups prefer the O-condensation (for example, 3,4,5-trimethoxyphenylethanal gives the hexamethoxylated Kagan's ether in 51% yield whereas 3,4-dimethoxyphenylethanal leads to the tetramethoxylated 2-phenylnaphthalene in 20% yield). However, using 2,3-dimethoxyphenylethanal, the two possible compounds may be obtained [8].

Arylethanal precursors were also subjected to acid treatment. Similarly to the above observations, phenylserine yields 2-phenylnaphthalene in good yield [7] whereas 3,4-dihydroxyphenylserine leads to the nitrogen Kagan's ether analogue [9]. Carter *et al.* [7] speculate that phenylethanal may be issued from a semipinacolic rearrangement of phenylserine, Fig. (**3**) but Fujiwara *et al.* [9] suggest an intramolecular decarboxylation/dehydration giving an enamine that can be partially hydrolysed to give 3,4-dihydroxyphenylethanal. A subsequent Pictet-Spengler cyclisation followed by an intramolecular Friedel-Crafts cyclisation give the nitrogen Kagan's ether analogue.

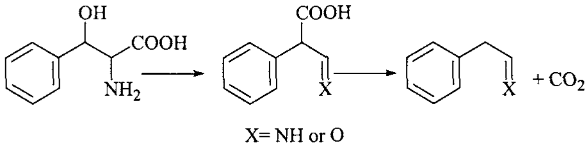



**Fig. (3).** Conversion of phenylserine into phenylethanal

More curiously, N-tosylated phenylalanine derivatives [10] and phenyllactic acid [11] can also be converted into 2-phenylnaphthalene in moderate yields whereas 3,4-dimethoxyphenyllactic acid treated with boron tribromide gives Kagan's ether in almost the same yield as 3,4-dimethoxyphenylethanal does [11]. In these cases, the decomposition of aryllactic acid or alanine derivatives may give arylethanal with the formation of water and carbon monoxide, Fig. (**4**).

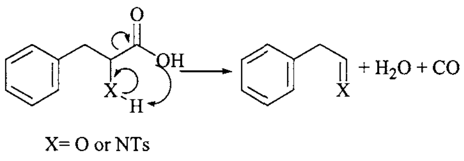



**Fig. (4).** Possible rearrangement of phenyllactic acid or alanine derivatives

There are few examples of natural 2-phenylnaphthalene derivatives. To our knowledge, the one natural 2-phenylnaphthalene derivative has been isolated from the Marine Sponge *Jaspis* sp. [12]. The authors postulated a biosynthesis from 3,4-dihydroxyphenylethanal, but C6-C3 derivatives, i.e., DOPA or 3,4-dihydroxyphenyllactic acid cannot be rejected considering the above discussion.

Kagan's ether structurally related compounds have not been found in Nature but their nitrogen analogues belong to the pavine alkaloid family. Pavinan and isopavinan alkaloids can be formally viewed as oxidatively cyclised 1-benzyl-1,2,3,4-tetrahydroisoquinolines and are most frequently obtained by acid-catalysed cyclisation of appropriately oxidised congeners of these isoquinolines [13]. Numerous attempts have been made to convert Kagan's ethers into pavine, but all were unsuccessful [4].

## ARYLACETONES

The acid-catalysed aldol condensation of phenylacetone has previously been reported by Cort et al [14]. They showed the formation of 1-benzyl-3-methylnaphthalene from phenylacetone and 70% sulfuric acid under reflux. This result was not surprising since unsymmetrical aliphatic ketones undergo self-condensation by attack of the carbonyl group on the less hindered α carbon atom. Moreover 4-methyl-1,5-diphenylpent-3-en-2-one was formed and cyclized to give the same product under strong acidic conditions [15]. Kagan et al [6] had also submitted phenylacetone to fluorosulfonic acid treatment and found only an electropilic substitution of the aromatic ring ortho and para by a fluorosulfonyl group. They did not explain the contrast between phenylethanal and phenylacetone and simply suggested a steric effect due to the additional substituent to the carbonyl function.

We found [16] that arylacetones treated with boron tribromide give the 1,3-dimethyl-2-phenylnaphthalenes in good yields with a concomitant demethylation when the aromatic ring is substituted by methoxy group(s). The mechanism is a tandem aldol condensation-intramolecular cyclisation with a high regioselectivity. In order to clearly investigate the scope of the reaction of arylacetones with borom tribromide, numerous arylacetones were tested [17]. It was concluded that:

(i) when the aromatic ring was unsubstituted or subsituted by an electron-donating group, 1,3-dimethyl-2-arylnaphthalenes were obtained in moderate to high yields

(ii) intramolecular cyclisations may occur when the aromatic ring is substituted by a methoxy group in position ortho leading either to intramolecular acetal or the 2-methylbenzofurans, Fig. (**5**)

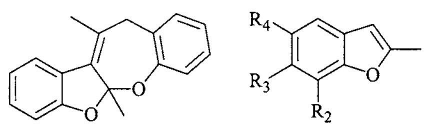



**Fig. (5).** Products of cyclisation observed in the case of 2-methoxyphenylacetones

(iii) bromination occurred when a phenanthrene ring was built, Fig. (**6**)

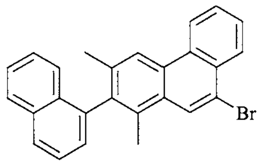



**Fig. (6).** Brominated product obtained in the case of the reaction of 1-naphthylacetone with boron tribromide

(iv) when the aromatic ring was substituted by an electron-withdrawing group, the intramolecular cyclisation was totally inhibited. The isolation of 4-methyl-3,5-bis(3-trifluoromethylphenyl)pent-2-en-3-one, Fig. (7) in the case of 3-trifluorophenylacetone suggested that the aldol condensation occurred but the intramolecular cylcisation was deactivated due to the presence of the trifluoromethyl group.

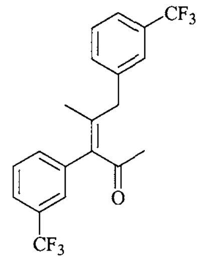



**Fig. (7).** 4-methyl-3,5-bis(3-trifluoromethylphenyl)pent-2-en-3-one

The cross-condensation, i.e., reaction of two different arylacetones, has been carried out using 3,4-dimethoxyphenylacetone and another variable arylacetone [18]. The objective was to obtain from only one experiment and after repeated chromatographies at least four different molecules that can be tested as HIV-1 integrase inhibitors. In effect, when 3,4-

dimethoxyphenylacetone was reacted with phenylacetone or arylacetone substituted by an electron-withdrawing group in the presence of boron tribromide, the four possible 1,3-dimethyl-2-arylnaphthalenes were isolated in modest yields (particularly the products of cross-condensation that are difficult to purify). As expected, 3,4-dimethoxyphenylacetone reacted with trifluorophenylacetones to give only two products, Fig. (**8**), i.e., the product of self-condensation of 3,4-dimethoxyphenylacetone and the compound due to the condensation of the enolborinate of trifluoromethylacetone with 3,4-dimethoxyphenylacetone. This last result confirms that the limiting step of this reaction is that of cyclisation.

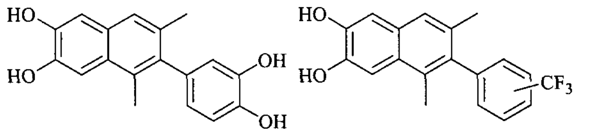



**Fig. (8).** the products of the reactions of 3,4-dimethoxyphenylacetone and trifluoromethylacetone in the presence of boron tribromide

To our knowledge, very few examples of natural products possessing a 1,3-dimethyl-2-phenylnaphthalene skeleton are known. The only ones are neolignans isolated from *Mentha haplocalyx* [19] and *Salvia officinalis* [20], Fig. (**9**).

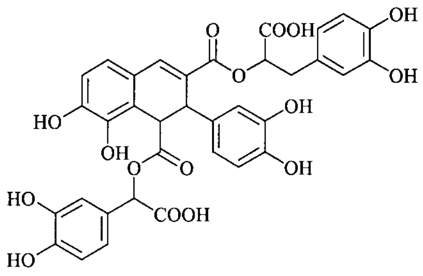



**Fig. (9).** Neolignan isolated form *Salvia officinalis*

It is probably biosynthesised from an isoferulic acid derivative [21] and its 1-decarboxy derivative has previously been obtained from the same intermediate as that of salvianolic acid F [22,23]. To date, attempts to synthetise it by a more straightforward strategy have failed. In order to obtain the correctly substituted 2-phenylnaphthalene skeleton, 2-bromo-

4,5-dimethoxyphenylacetone was prepared and treated in acidic conditions ($BBr_3$ or HCl), but the aldol condensation failed. Nevertheless, the 2-phenylnaphthalene skeleton of these neolignans can be built using the intramolecular Diels-Alder cyclisation strategy [22].

## ARYLPYRUVIC ACIDS

Lignans are dimers of phenylpropanoid units linked by the central carbons of their side chains while naturally occurring dimers that exhibit linkages other than this type of bond are known as neolignans. Much attentions has been devoted to the oxidative, acidic or alkaline dimerisations of arylpropenoid molecules and particularly 4-hydroxycinnamic acids such as ferulic and caffeic acids [24-34].

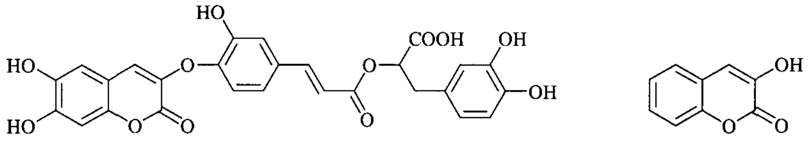



**Fig. (10).** Sagecoumarin (left) and 3-hydroxycoumarin (right)

The recent isolations of sagecoumarin [35] , Fig. **(10)** (a 3-aryloxy-6,7-dihydroxycoumarin) and 3-hydroxycoumarin [36] led us to investigate their possible obtention from conveniently substituted arylpyruvic acids. Since the dimerisation of arylpyruvic acid has been proposed in the biosynthesis of butyrolactone isolated from *Aspergillus terreus* [37], Fig. **(11)**, we developed an interest in the potential acid-catalysed dimerisation of these molecules.

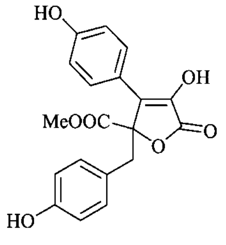



**Fig. (11).** Butyrolactone II isolated from *Aspergillus terreus*.

In connection with our studies on the dimerisation of aylethanals and arylacetones with boron tribromide, we first turn to the reaction of methoxylated arylpyruvic acids [38]. Arylpyruvic acids that were obtained mainly in their Z-enol form, did not dimerise. When the ortho aromatic position is unsubstituted, only an isomerisation occurs that allowed us to isolate some 2-hydroxyarylpropenoic acids in their E-form. In the cases of (2-methoxyphenyl)arylpropenoic acids, cyclisations were also observed leading of mixtures of benzofuran-2-carboxylic acids and 3-hydroxycoumarins that are easily separated, Fig. (**12**). We observed a competition between cyclisation and isomerisation that have apparently similar kinetic parameters.

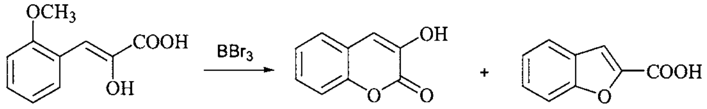



**Fig. (12).** Reaction of 2-methoxyphenylpyruvic acid with boron tribromide

In connection with our interest in the synthesis of salvianolic acids [22,23], we needed aryllactic acids that are usually obtained by zinc amalgam reduction in boiling hydrochloric acid [39]. Serendipitously, we isolated, along with the expected arylllactic acids, 2-hydroxyfuran-2-ones, Fig. (**13**).

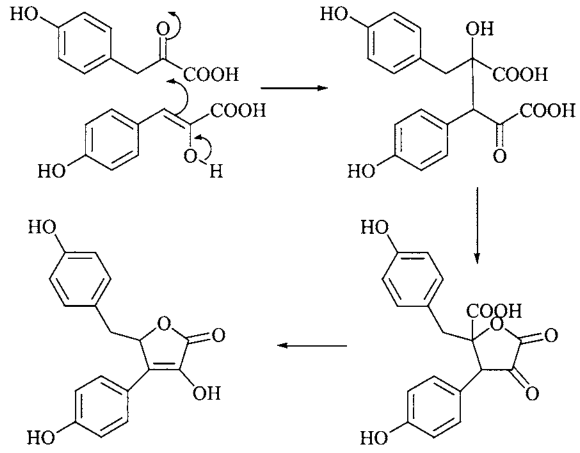



**Fig. (13).** Dimerisation of 4-hydroxyphenylpyruvic acid in concentrated hydrochloric acid.

The presence of zinc amalgam is not necessary to promote the dimerisation and 2-hydroxyfuran-2-ones can also be obtained from arylpyruvic acids. The supposed mechanism of formation of these compounds involves an aldol condensation followed by a lactonisation and finally a decarboxylation [40].

## DIARYLETHANOID DERIVATIVES

Numerous natural polyphenols such as moracins [41-43] or norlignans [44], Fig. (**14**) contain a 2-phenylbenzofuran unit that has stimulated numerous studies [45] on the synthesis of this skeleton.

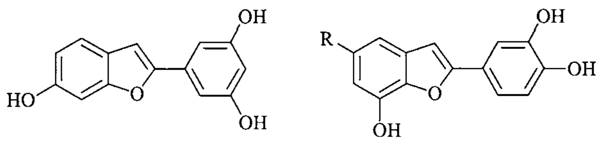



**Fig. (14).** Moracin C (left) and norlignans (right)

The fourth part of this review concerns the development of the previously described intramolecular cyclisation of 2-methoxyphenyl acetones and 2-hydroxy-3-(2-methoxyphenyl)propenoic acids leading to 2-substituted benzofurans [17, 38].

The acid-catalysed tandem deprotection-cyclisatio of diarylethanoid units is well documented [46-49] but the reported procedure generally requires hard conditions (concentrated acid, high temperature) and gives variable yields. The synthesis of polyhydroxylated 2-phenylbenzofurans from 1,2-diarylethanoid units and boron tribromide may be of particular interest due to the limited number of steps involved (five steps from the commercially available benzylalcohols), its simplicity and the fact that exhaustive purifications of the intermediate are unnecessary of exhaustive purifications of the intermediates. In a first step, three 1,2-diarylethanoid units were synthesised in order to design the best model allowing cyclisation, Fig. (**15**).

Best results were obtained with the epoxide and the alcohol ester and therefore the mixture of the products of oxidation of the stilbenes with m-chloroperbenzoic acid was used.

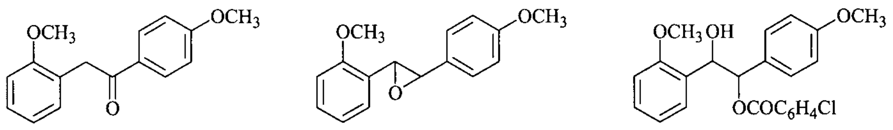
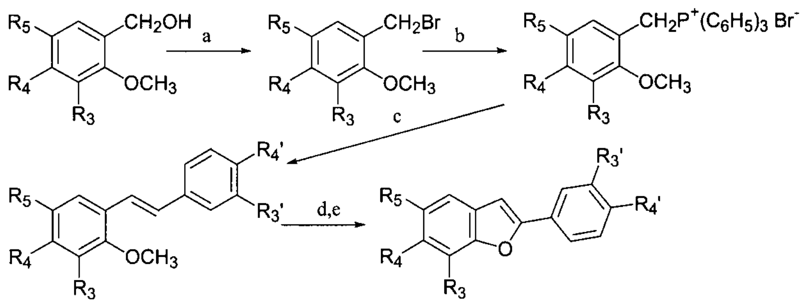



**Fig. (15).** 2-(2-methoxyphenyl)1-(4-methoyxphenyl)ethanone (left), 1,2-epoxy-1-(2-methoxyphenyl)-2-(4-methoxyphenyl)ethane (center) and 2-(3-chlorobenzoyloxy)-1-(2-methoxyphenyl)-2-(4-methoxyphenyl)ethanol (right)

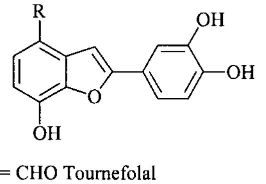
Using this procedure, Fig.(**16**), seven hydroxylated 2-arylbenzofurans were obtained in 17-57% overall yields (from the corresponding benzyl alcohols) in only five steps [50].




a. HBr 48% or $PBr_3$, 88-96%; b. $P(C_6H_5)_3$, toluene, reflux, 4h, 75-90%; c. MeOH, MeONa, then 4-methoxy or 3,4-dimethoxybenzaldehyde, 76-96%; d. mcpba, $CH_2Cl_2$, $NaHCO_3$; e. $BBr_3$, $CH_2Cl_2$, 20°C, 21-66%

**Fig. (16).** Synthesis of 2-arylbenzofurans from benzylalcohols

We are currently attempting to synthesise [51] the new antilipoperoxidant natural tournefolal and tournefolic acid [52], Fig. (**17**) using this procedure as a key step in the building of benzofuran skeleton.




R= CHO Tournefolal
R= CH=CHCOOH Tournefolic acid A

**Fig. (17).** Tournefolal and tournefolic acid

## BIOLOGICAL ACTIVITIES

Due to the wide structural diversity, natural polyphenols possess a broad range of biological activities. Nevertheless, natural (as well as synthetic) polyphenols are mainly known for their anti-tumour, antimitotic, antiviral and antiinflammatory activities, sometimes associated with antioxidant properties. The involvement of free radicals in the pathology of human diseases (i.e., intestinal diseases, atherosclerosis, reprfusion injuries, cardiac diseases, neurodegeneration, respiratory disorders, inflammation, diabetes, cancer and aging) justifies the use of natural antioxidants or synthetic pharmacological agents with antioxidant activity.

### Antioxidant properties

For about ten years, I have been interested in the antioxidant properties of polyphenol derivatives, i.e., interaction with various reactive oxygen species such as hydroxyl radical (OH°) superoxide ($O_2^{-\circ}$) as well as their enzymatic source (xanthine oxidase)[53-56].

Polyphenols bearing two hydroxyl groups in ortho or para positions are known to oxidize readily into a radicalar form called semiquinonic radical. This kind of radical is relatively stable but may generate highly toxic species thus limiting its usefulness as an antioxidant. For this reason, we recently developed a new strategy in the design of lipophilic antioxidant.

This new strategy consists of the synthesis of molecules that possess hydroxyl groups in such positions that a radical species can be stabilized by mesomery. This feature is found in natural flavonol such as kaemferol that possesses undeniable antioxidant properties. The first published example is a lipophilic analogue of vitamin C, Fig (**13**) [40]. In this paper, we have proved that the synthesised substituted 2-hydroxyfuran-2-one is a true ascorbic acid analogue. A radical anion that gives very similar data has been generated under the same conditions as for ascorbic acid with a stability somewhat lower and a redox potential lower than those of ascorbyl radical. Its antioxidant properties are also similar to that of ascorbic acid but it inhibits LDL peroxidation induced by $Cu^{2+}$ or AAPH more efficiently probably due to a higher lipophilicity.

3-Hydroxycoumarins, Fig. (**12**), were also investigated for their potential to generate stable radicals [57] and are currently being studied for their antioxidant properties.

Day by day, new natural polyphenols are being isolated and are revealing potent antioxidant properties. Particularly in the field of nutriments, due to powerful lobbies, exagerate conclusions have been drawn such as "wine is good for your health". Nevertheless, reequilibrating our nutrition by increasing fruit and vegetables is expected to be healthy by increasing the assimilation of numerous biologically active polyphenols.

## Antiinflammatory activities

Leukotrienes are potent mediators of inflammation derived from arachidonic acid through the action of 5-lipoxygenase enzyme (5-LO) [58]. The peptidoleukotrienes, $LTC_4$, $LTD_4$ and $LTE_4$ are powerful spasmogens, which have been implicated in the inflammation and allergy processes [59]. Thus, the control of leukotriene biosynthesis through the inhibition of 5-LO represents a potential new method for the treatment of diseases such as asthma or rheumatoid arthritis [60]. Among the known inhibitors of the 5-LO are a variety of polyhydroxylated natural products such as caffeic acid [61], NDGA [62] and flavonoids [63]. The rational design of new drugs requires the maximum knowledge of the target itself (structure, role, mechanism of the 5-LO) and the action mechanism of the known inhibitors [64]. In the case of 5-LO, answers to most of the fundamental structural and mechanistic questions have remained elusive due to the lack of an atomic resolution model. Most of our knowledge about lipoxygenases is based upon studies on soybean lipoxygenase-1 which is closely related to mammalian lipoxygenases [65-67]. Some polyhydroxylated 2-phenylnaphthalenes and the oxy analogue of tetrahydroxypavinan have been tested for their ability to inhibit the 5-lipoxygenase in order to gain an insight into the flexibility of the natural substrate, arachidonic acid, for its access to iron (III) ion in the active site of the enzyme. We have shown that three compounds, fig (**18**) as well as NDGA are potent iron (III) reductors, inhibit free radicals such as DPPH efficiently, give very stable semiquinonic radicals and finally are potent redox inhibitors of 5-lipoxygenase.

The fact that the totally rigid and bulky epoxydibenzocyclooctene is only 2.5 times less active than NDGA suggests that the cavity devoted to the approach of arachidonic acid is sufficiently flexible to accept bulky inhibitors.

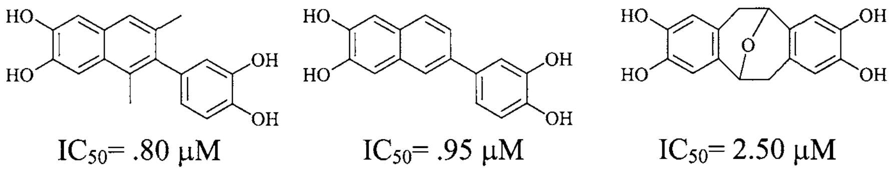
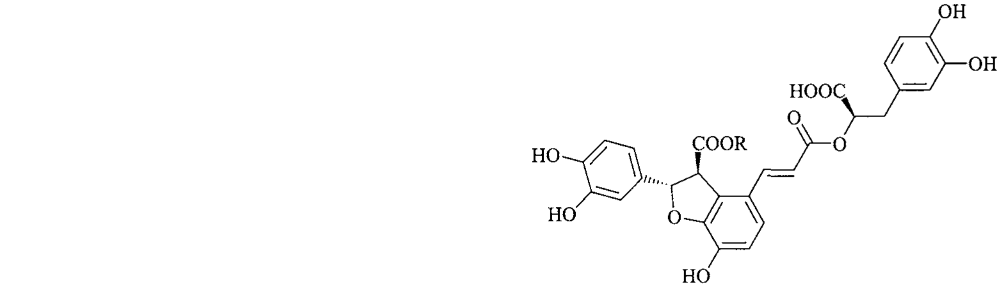



**Fig. (18).** 5-Lipoxygenase inhibitors

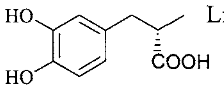
## Anti-HIV activity

The acquired immunodeficiency syndrome (AIDS) which is the final and most serious stage of human immunodeficiency virus (HIV) infection, renders the body susceptible to a variety of normally manageable infections, cancer and other diseases [68-71]. Reverse transcriptase, protease and integrase are three enzymes required in the HIV replication cycle. HIV integrase (IN) is currently recognized as an attractive target against AIDS. It catalyses the integration of viral DNA into host DNA in two steps: 3'-processing and strand transfer. First, integrase cleaves the last two nucleotides from each 3'-end of the linear viral DNA. The subsequent DNA strand transfer reaction involves the nucleophilic attack of these 3'-ends on host chromosomal DNA. A number of natural polyphenols have been reported recently to inhibit HIV-1 integrase in biochemical assays [72-79]. The most potent compounds tend to contain multiple aromatic rings and a catechol moiety.

They are generally active at the two steps of the integration but are not selective for IN and the practical utility of those catechol-containing inhibitors is severely reduced by cytotoxicity even though they have been found to inhibit HIV-1 integrase in vitro. Nevertheless, lithospermic acids, Fig. (**19**) were found to be active on purified IN as well as on HIV-1 infected H9 cells, and showed no cytotoxicity to H9 cells [80].




R= H :Lithospermic acid A

R= 

Lithospermic acid B

**Fig. (19).** New non-toxic HIV-1 integrase inhibitors

Numerous natural polyphenols were also found to be potent anti-HIV agents but their exact targets in the viral replication process were not always determined, Fig (**20**)[81-82].

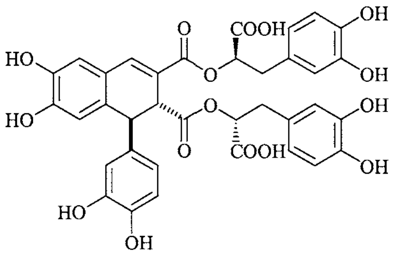
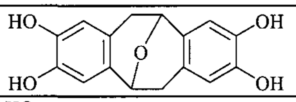
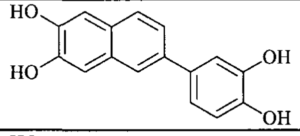
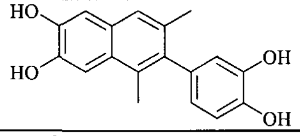
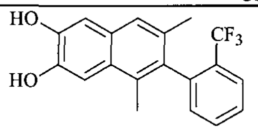
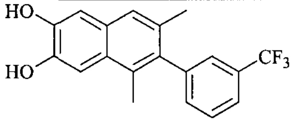
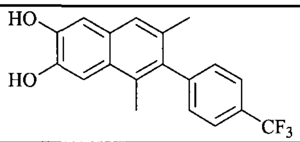



**Fig. (20).** Rabdosiin

Amongst fourteen tested polyphenols [18], we have found six active molecules (table 1) against purified HIV-1 IN, two of which are active at micromolar concentrations and the four others between 13 and 23 μM. Against infected CEM cells, five of them were found to be active at ten micromolar $IC_{50}$ but they also exibited a weak to relatively high toxicity. We are currently studying to docking of the rigid 5,6,13,14-Tetrahydroxy-1,2,9,10-tetrahydro-1,9-epoxydibenzo[a,e]cyclooctene to the HIV-1 IN catalytic core. The trifluoromethyl group may be useful to maintain the HIV-1 IN inhibitor activity, increase the antiviral activity and decrease the toxicity. Other polyphenols possessing the rigid structure as well as

the trifluoromethyl pharmacophore are currently tested as HIV-1 IN inhibitors.

**Table 1.** **New HIV-1 IN and antiviral inhibitors**

| Tested molecules | HIV-1 IN activity $IC_{50}$ in μM | Antiviral activity $IC_{50}$ in μM | Cytotoxicity $TC_{50}$ in μM |
|---|---|---|---|
|  | 1.3 | 98 | Not Reached |
|  | 2.1 | 9.9 | 51 |
|  | 23 | 8.7 | 33 |
|  | 22 | 10.3 | 13.6 |
|  | 13 | 5.7 | 72.6 |
|  | 21 | 10.5 | 19 |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## REFERENCES


[1] Nielsen, A.T.; Houlihan, W.J. in *Organic Reactions*, John Wiley and Sons, Inc.: New York, **1968**, Vol. *16*, 1-438.

[2] Jung, M. E.; Mossman, A. B.; Lyster, M. A.; *J. Org. Chem.* **1978**, *43*, 3698-3701.

[3] Dupont, R.; Cotelle, P.; *Tetrahedron Lett.*, **1998**, *39*, 8457-8460.

[4] Jung, M. E.; Miller, S. J.; *J. Amer. Chem. Soc.* **1981**, *103*, 1984-1992.

[5] Kagan, J.; Chen, S. Y.; Agdeppa, D. A.; Watson, W. H.; Zabel, V. *Tetrahedron Lett.*, **1977**, *51*, 4469-4470.

[6] Kagan, J.; Agdeppa, D. A.; Chang, A. I.; Chen, S. A.; Harmata, M. A.; Melnick, B.; Patel, G.; Poorker, C.; Singh, S. P.; Watson, W. H.; Chen, J. S.; Zabel, V.; *J. Org. Chem.*, **1981**, *46*, 2916-2930.

[7] Carter, H. E.; Van Loon, E. J.; *J. Amer. Chem. Soc.*, **1938**, *60*, 1077-1080.

[8] Maurin, C.; Cotelle, P.; To be published

[9] Fujiwara, N.; Ueda, Y.; Ohashi, N.; *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 743-748.

[10] Seong, M. R.; Song, H. N.; Kim, J. N.; *Tetrahedron Lett.*, **1998**, *39*, 7101-7104.

[11] Cotelle, P. Unpublished results

[12] Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N.; *Tetrahedron*, **1994**, *50*, 13583-13592.

[13] Rice, K. C.; Ripka, W. C.; Reden, J.; Brossi, A.; *J. Med. Chem.*, **1980**, *45*, 601-607.

[14] Cort, L.A.; Manders, R.G.; Parlett, G.R. *J. Chem. Soc.* **1964**, 2844.

[15] Colonge, J. *Bull. Soc. Chim. Fr.* **1931**, *49*, 426-441.

[16] Cotelle, P.; Catteau, J.P. *Tetrahedron Lett.*, **1997**, *38*, 2969-2972.

[17] Dupont, R.; Cotelle, P. *Synthesis*, **1999**, 1651-1655.

[18] Dupont, R.; Jeanson, L.; Mouscadet, J.F.; Cotelle, P. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 3175-3178.

[19] Matano, Y.; Watano, T.; Oono, A.; Kanita, M.; Saito, J.; Suzuki, A. **1993**, Jpn Kokai Tokkyo Koho, JP 05 25,082 [CA: 118: 240926e].

[20] Lu, Y.; Foo, L.Y. *Tetrahedron Lett.*, **2001**, *42*, 8223-8225.

[21] Cotelle, P.; Vezin, H. *Tetrahedron Lett.*, submitted

[22] Dalla, V.; Cotelle, P. *Tetrahedron Lett.*, **1998**, *39*, 8285-8286.

[23] Dalla, V.; Cotelle, P. *Tetrahedron*, **1999**, *55*, 6923-6930.

[24] Ahmed, R.; Lehrer, M.; Stevenson, R. *Tetrahedron*, **1973**, *29*, 3753-3759.

[25] Utley, J.H.P.; Gullu, M.; Motevalli, M. *J. Chem. Soc. Perkin Trans. 1*, **1995**, 1961-1970.

[26] Grumbinger, H.G.; Vahlensieck, U.; Winterhoff, H. *Planta Med.* **1993**, *59*, 491-493.

[27] Pieters, L.; VanDyck, S.; Gao, M.; Bai, R.; Hamel, E.; Vlietinck, A.; Lemière, G. *J. Med. Chem.* **1999**, *42*, 5475-5481.

[28] Wasserman, H.H.; Brunner, R.K.; Buynak, J.D.; Carter, C.G.; Oku, T.; Robinson, R.P. *J. Amer. Chem. Soc.* **1985**, *107*, 519-521.

[29] Miller, I.J. *Tetrahedron Lett.* **1972**, 4955-4958.

[30] Eskins, K.; Glass, C.; Rohwedder, W.; Kleiman, R.; Sloneker, J. *Tetrahedron Lett.* **1972**, 861-864.
[31] a) Iguchi, M.; Nishiyama, A.; Hara, M.; Terada, Y.; Yamamura, S. *Chem. Lett.* **1978**, 1015-1018. b) Iguchi, M.; Nishiyama, A.; Eto, H.; Terada, Y.; Yamamura, S. *Chem. Lett.* **1979**, 1397-1400.
[32] Fulcrand, H.; Cheminat, A.; Brouillard, R.; Cheynier, V. *Phytochemistry*, **1994**, *35*, 499-505.
[33] Cotelle, P.; Vezin, H. *Tetrahedron Lett.*, **2001**, *42*, 3303-3305.
[34] Cilliers, J.J.L.; Singleton, V.L. *J. Agric. Food Chem.* **1991**, *39*, 1298-1303.
[35] Lu, Y.; Foo, L.Y.; Wong, H. *Phytochemistry*, **1999**, *52*, 1149.
[36] Macias, F.A.; Simonet, A.M.; Galindo, J.C.G.; Castellano, D. *Phytochemistry*, **1999**, *50*, 35
[37] a) Kiriyama, N.; Nitta, K.; Sakagushi, Y.; Taguchi, Y.; Yamamoto, Y. *Chem. Pharm. Bull.* **1977**, *25*, 2593; b) Nitta, K.; Fujita, N.; Yoshimura, T.; Arai, K.; Yamamoto, Y. *Chem; Pharm. Bull.* **1983**, *31*, 1528
[38] Dupont, R.; Cotelle, P. *Tetrahedron Lett.*, **2001**, *42*, 597-600.
[39] Wong, H.N.C.; Xu, Z.L.; Chang, H.M.; Lee, C.M. *Synthesis*, **1992**, 793
[40] Cotelle, P.; Cotelle, N.; Teissier, E.; Vezin, H. *Bioorg. Med. Chem.*, **2003**, in press
[41] Takasugi, M.; Nagao, S.; Masamune, T.; Shirata, A.; Takahashi, K. *Tetrahedron Lett.*, **1978**, *9*, 797-798.
[42] Takasugi, M.; Nagao, S.; Ueno, S.; Masamune, T.; Shirata, A.; Takahashi, K. *Chem. Lett.*, **1978**, 1239-1240.
[43] Takasugi, M.; Nagao, S.; Masamune, T.; Shirata, A.; Takahashi, K. *Tetrahedron Lett.* **1979**, *10*, 4675-4678.
[44] Ward, R. S. *Nat. Prod. Rep.*, **1999**, *16*, 75-96 and previous reports referred to herein.
[45] Mc Allister, G. D.; Hartley, R. C.; Dawson, M. J.; Knaggs, A. R. *J. Chem. Soc. Perkin Trans 1*, **1998**, 3453-3457 and references cited therein.
[46] Whalley, W. B.; Lloyd, G. *J. Chem. Soc.*, **1956**, 3213-3224.
[47] Bowyer, W. J.; Robertson, A.; Whalley, W. B. *J. Chem. Soc.*, **1957**, 542-543.
[48] Moureu, H.; Chovin, P.; Sabourin, R.; Ducros, M. *Bull. Soc. Chem. Fr.*, **1956**, 301-306.
[49] Fumiyuki, K.; Xing, C.; Yoshisuke, T. *Heterocycles*, **1990**, *31*, 629-636.
[50] Dupont, R.; Cotelle, P. *Tetrahedron*, **2001**, *57*, 5585-5589.
[51] Bailly, F.; Cotelle, P. to be published
[52] Lin, Y.L.; Chang, Y.Y.; Kuo, Y.H.; Shiao, M.S. *J. Nat. Prod.*, **2002**, *65*, 745-747.
[53] Capelle, S.; Planckaert, B.; Cotelle, P.; Catteau, J.P. *J. Chim. Phys.*, **1992**, *89*, 561-566.
[54] Capelle, S.; Cotelle, N.; Cotelle, P.; Bernier, J.L.; Catteau, J.P. *Redox Report*, **1994**, *1*, 131-137.
[55] Grenier, J.L.; Cotelle, N.; Cotelle, P.; Catteau, J.P. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 431-434.
[56] Dupont, R.; Goossens, J.F.; Cotelle, N.; Vrielynck, L.; Vezin, H.; Hénichart, J.P.; Cotelle, P. *Bioorg. Med. Chem.*, **2001**, *9*, 229-235.

[57] Vezin, H.; Cotelle, P. *Res. Chem. Intermed.*, **2003**, in press
[58] a. Musser, J.H.; Kreft, A.F. *J. Med. Chem.*, **1992**, *35*, 2501-2524; b. Brooks, C.D.W.; Summers, J.B. *J. Med. Chem.*, **1996**, *39*, 2629-2654.
[59] a. Samuelsson, B. *Science*, **1983**, *220*, 568-575; b. Lewis, R.A.; Austen, K.F. *Nature*, **1981**, *293*, 103-108.
[60] a. Shaw, A.; Krell, R.D. *J. Med. Chem.*, **1991**, *34*, 1235-1242; b. Mc Millan, R.M.; Walker, E.R.H. *Trends Pharm. Sci.*, **1992**, *13*, 323-330.
[61] Koshihara, Y.; Neichi, T.; Murota, S.I.; Lao, A.N.; Fujimoto, Y.; Tatsuno, T. *Biochim. Biophys. Acta*, **1984**, *792*, 92-97.
[62] Yoshimoto, T.; Furukawa, M.; Yamamoto, S.; Horie, T.; Watanabe-Kohno, S. *Biochem. Biophys. Res. Commun.*, **1983**, *116*, 612-618.
[63] Baumann, J.; Von Bruchhausen, F.; Wurm, G. *Prostaglandins*, **1980**, *20*, 627-639.
[64] Young, R.N. *Eur. J. Med. Chem.*, **1999**, *34*, 671-685.
[65] Kemal, C.; Louis-Flamberg, P. L.; Krupinski-Olsen R.; Shorter, A. L. *Biochemistry*, **1987**, *26*, 7064-7072.
[66] Nelson, M. J. *Biochemistry*, **1988**, *27*, 4273-4278.
[67] Boyington, J. C.; Gaffney, B. J.; Amzel, L. M. *Science*, **1993**, *260*, 1482-1486.
[68] Gait, M.J.; Karn, *J. Trends. Biotechnol.*, **1995**, *13*, 430-438.
[69] Robinson, W.E. *J. Infect. Med.*, **1998**, *15*, 129-137.
[70] Andrake, M.D.; Skalka, A.M. *J. Biol. Chem.*, **1996**, *271*, 19633-19636.
[71] Pommier, Y.; Marchand, C.; Neamati, N. *Antiviral Res.*, **2000**, *47*, 139-148.
[72] Farnet, C.M.; Wang, B.; Hansen, M.; Lipford, J.R.; Zalkow, L.; Robinson, W.E.; Siegel, J.; Bushman, F. *Antimicrob. Agents Chemother.*, **1998**, *42*, 2245-2253.
[73] Lin, Z.; Neamati, N.; Zhao, H.; Kiryu, Y.; Turpin, J.A.; Aberham, C.; Strebel, K.; Kohn, K.; Witvrouw, M.; Pannecouque, C.; Debyser, Z.; De Clercq, .; Rice, W.G.; Pommier, Y.; Burke, T.R. *J. Med. Chem.*, **1999**, *42*, 1401-1414.
[74] Fesen, M.R.; Pommier, Y.; Leteurtre, F.; Hiroguchi, S.; Yung, J.; Kohn, K. *Biochem. Pharmacol.*, **1994**, *48*, 595-608.
[75] Kim, H.J.; Woo, E.R.; Shin, C.G.; Park, H.A. *J. Nat. Prod.*, **1998**, *61*, 145-148
[76] Eich, E.; Pertz, H.; Kaloga, M.; Schulz, J.; Fesen, M.R.; Mazumder, A.; Pommier, Y. *J. Med. Chem.*, **1996**, *39*, 86-95.
[77] Mazumder, A.; Neamati, N.; Sunder, S.; Schulz, J.; Pertz, H.; Eich, E.; Pommier, Y. *J. Med. Chem.*, **1997**, *40*, 3057-3063.
[78] Singh, S.B.; Jayasuriya, H.; Salituro, G.M.; Zink, D.L.; Shafiee, A.; Heimbuch, B.; Silverman, K.C.; Lingham, R.B.; Genilloud, O.; Teran, A.; Vilella, D.; Fellock, P.; Hazuda, D. *J. Nat. Prod.*, **2001**, *64*, 874-882.
[79] Singh, S.B.; Zink, D.L.; Heimbuch, B.; Genilloud, O.; Teran, A.; Silverman, K.C.; Lingham, R.B.; Fellock, P.; Hazuda, D. *Org. Lett.*, **2002**, *4*, 1123-1126.
[80] Abd-Elazem, I.; Chen, H.S.; Bates, R.B.; Huang, R.C.C. *Antiviral Res.*, **2002**, *55*, 91-106.
[81] Gnabre, J.; Huang, R.C.C.; Bates, R.B.; Burns, J.J.; Caldera, S.; Malcomson, M.E.; McClure, K.J. *Tetrahedron*, **1995**, *51*, 12203-12210.
[82] Kashiwada, Y.; Nishizawa, M.; Yamagishi, T.; Tanaka, T.; Nonaka, G.I.; Cosentino, L.M.; Snider, J.V.; Lee, K.H. *J. Nat. Prod.*, **1995**, *58*, 392-400.

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# LABIATAE FLAVONOIDS AND THEIR BIOACTIVITY

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**ABSTRACT**: There are about 200 genera in Labiatae family in the world, whereas in Turkish flora 45 genera are present. A literature survey revealed that only 10 genera are extensively investigated for the flavonoidal compounds, some work were done about 23 genera, but the rest had no flavonoidal investigation. The biological activities of flavonoids were also studied together with their structural studies. The most studied genus for the biological activities is *Scutellaria*. Baicalein, baicalin and wogonin showed a number of important activities.

## INTRODUCTION

Flavonoids are found throughout the plant kingdom with the exception of most algae [1]. In Labiatae (Lamiaceae) family they occur in every part of the plants as in other families, but are usually found in the aerial parts. Flavonoids are biogenetically formed from a malonate-derived $C_6$ unit and a shikimic acid-derived $C_6$-$C_3$ phenylpropanoid moiety to give initially chalcones. The chalcone-flavanone isomeric pair then undergoes further transformations including oxidations, rearrangements, alkylations, acylations, and glycosylations all of which give structural diversity to the thousands of distinct flavonoids known at the present time [2], Fig.(**1**).

### Isolation and Purification of Flavonoids

Separation and purification of the flavonoids is a tedious work. For the rough separation of flavonoid mixture polyamide (polyclar), and silica gel columns could be used, also magnesol, cellulose powder, charcoal and

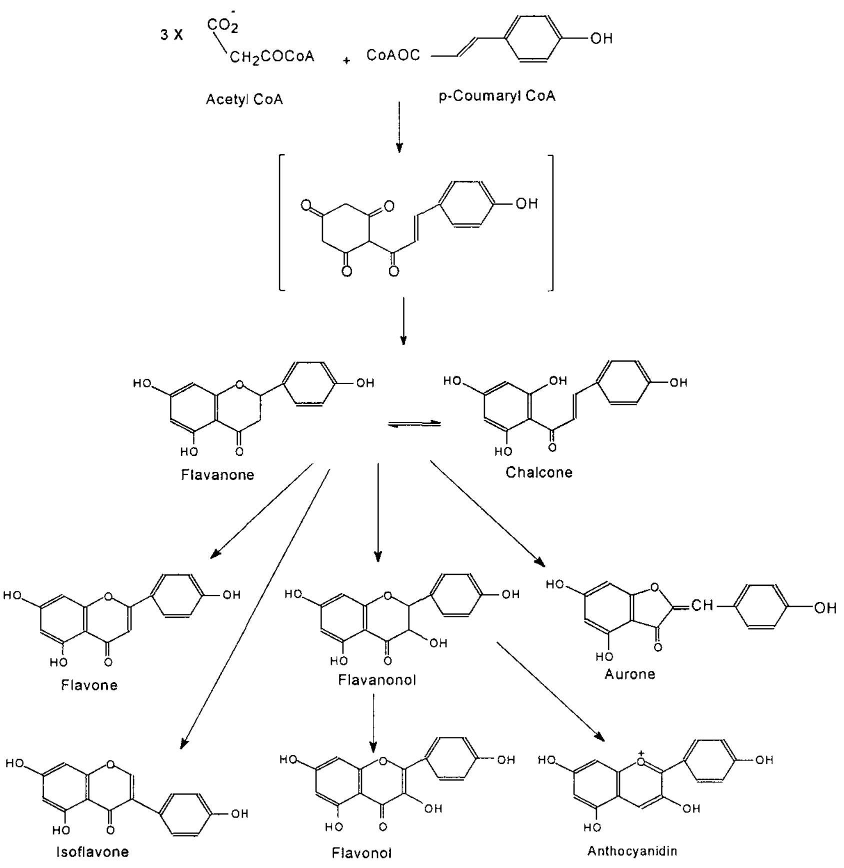



**Fig. (1)** Biogenesis of flavonoids

starch were tried successfully by some authors [3]. Elution of the columns are started with water than 20% MeOH is added, the amount of MeOH increases slowly up to 100%. The bands are detected on UV lights. Each collected fraction (150 ml) is subsequently analysed by two dimentional paper chromatography [4]. Sephadex LH-20 is used for the final cleaning of flavonoids, small columns (1x20 cm) are eluted with MeOH, and the bands are followed under UV light.

*Identification of Flavonoids*

Spectral methods are used for the identification of the flavonoids, since their amounts usually are very little, UV spectra is one of the most important tool. $^{1}H$ and $^{13}C$ NMR give very good information and MS

analysis establishes the structure. Sugar moieties of flavone glycosides could be decided by acidic and/or enzymatic hydrolysis.

*Acidic hydrolysis:* 2-5 mg of flavone glycoside dissolved in minimum amount of MeOH and mixed with 5% aqueous hydrochloric acid (1ml) and refluxed on an electrical heater for an hour. After cooling, the mixture is extracted with ether-EtOAc for the aglycone. The aqueous residue evaporated to dryness for the sugars. The EtOAc extract after drying and evaporation yields the aglycone.

*Enzymatic hydrolysis:* Since most of the flavone glycosides contain $\beta$-D-glucoside, for the hydrolysis of sugar the most common and widely used enzyme is $\beta$-glucosidase. The flavone glucoside (1-2 mg) dissolved in 2 ml of pH 5 buffer (an aqueous 0.5 M NaOAc solution adjusted to pH 5 with HOAc) and about 1 mg of enzyme ($\beta$-glucosidase) is added. The mixture is allowed to stand over night at $37^0$. When the solution is concentrated under high vacuum, and paper or thin layer chromatographed only the aglycone is detected.

### *Identification of Sugars*

The aqueous residue of the acid hydrolysed flavone glycoside could either be identified by paper or thin layer chromatographic analysis by comparing with the standard samples or after preparing their trimethylsilyl derivatives they were subjected gas chromatographic separations, and measuring the retention times will let us know the identity of the sugars [4, p.24-32].

### *Ultraviolet Spectral Analysis of Flavonoids* [4, p. 35-61]

For the UV spectra of flavonoids one needs the following reagents;

*Sodium methoxide* (NaOMe): Freshly cut metalic sodium (2.5 g) is added in small portions to dry spectroscopic grade methanol (100 ml).

*Aluminum chloride* ($AlCl_3$): Five grams of fresh anhydrous reagent grade $AlCl_3$ are added cautiously to spectroscopic grade methanol (100 ml).

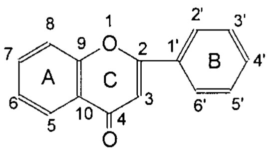
*Hydrochloric acid* (HCl): Concentrated reagent grade HCl (50 ml) is mixed with distilled water (100 ml) the solution is stored.

*Sodium acetate* (NaOAc): Anhydrous powdered reagent grade NaOAc is used.

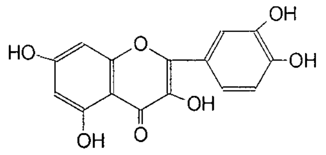
*Boric acid* ($H_3BO_3$): For Procedure I; Anhydrous powdered reagent grade $H_3BO_3$ is used.
For Procedure II: Spectroscopic grade MeOH (100 ml) is saturated with anhydrous reagent grade $H_3BO_3$.

## *The UV Spectra of Flavones and Flavonols in Methanol*

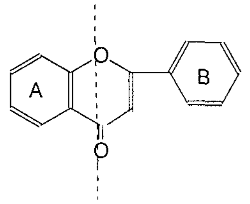
The MeOH spectra of flavones and flavonols exhibit two major absorption peaks in the region 240-400 nm. They are referred as Band I (usually 300-380 nm) and Band II (usually 240-280 nm). Band I is considered to be associated with absorption due to the B-ring cinnamoyl system and Band II with the absorption of the A-ring benzoyl system, **Fig.(2)** [4, p. 41-61].




Flavone skeleton




Flavonol skeleton




Benzoyl Cinnamoyl

**Fig. (2)**

## *UV Spectra in the Presence of NaOMe*

Sodium methoxide being a strong base ionizes all hydroxyl groups on the flavonoid nucleus. However on the UV spectra of flavones and flavonols, it is used for the detection of free 3- and/or 4'-hydroxyl groups. Into the MeOH solution of the flavones and flavonols, the addition of NaOMe causes bathochromic shift in all bands, but in band I about 40-65 nm without decrease in the intensity which is diagnostic for a free 4'-OH

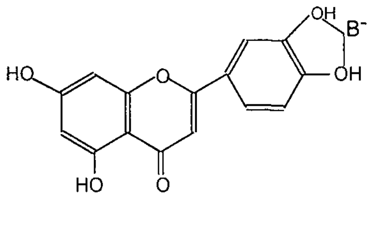
group. Although flavonols lacking a free 4'-OH group also gives 50-60 nm bathochromic shift in band I, there is usually a decrease in the intensity of the peak. The bathochromic shift results from the presence of a free 3-OH group. When there is a free 4'-OH group in a free 3-OH containing flavonols, the spectrum recorded with NaOMe degenerates in a few minutes. Flavonols which contain 3,3',4'-trihydroxyl system decompose faster than those having 3,4'-dihydroxyl system even with NaOAc.

*UV Spectra in the Presence of NaOAc*

Flavones and flavonols with a free 4'-OH group and no free 3- or 7-OH groups usually show a pronounced shoulder on the long wave side of Band I with the addition of NaOAc (not fused). If fused NaOAc (HOAc free) is added Band I shift is also observed, when 7-OH group is free whether or not containing a free 4'-OH group. Band I appears as a peak similar to that observed with NaOMe. Only when the compound posseses 5,6,7; 5,7,8 or 3,3',4'hydroxyl groups even if 3' is a methoxyl, the spectrum decomposes, therefore it is difficult to measure the presence or absence of free 7-OH groups unless the spectrum is measured immediately after the addition of the NaOAc to the cuvette.

*Detection of Ortho-Dihydroxyl Groups by Effect of NaOAc/$H_3BO_3$*

In the presence of NaOAc, boric acid will chelate with ortho-dihydroxyl groups, at all locations on the flavonoid nucleus, except at C-5,6. Flavones and flavonols containing a B-ring ortho-dihydroxyl group show a consistent 12-30 nm bathochromic shift of Band I in the presence of NaOAc/$H_3BO_3$ (see **I**).




**I**

In the A ring 6,7 and 7,8 dihydroxyl groups are also detectable under the same conditions.

*UV Spectra in the Presence of $AlCl_3$ and $AlCl_3/HCl$*

When there is free C-3 or C-5 hydroxyl groups the flavones or flavonols form acid stable complexes with the addition of $AlCl_3$. In addition, they form acid labile complexes with ortho-dihydroxyl groups. The $AlCl_3$ complex between C-4 keto function and either C-3 or C-5 hydroxyl group is stable in the presence of acid, Fig.(**3**).

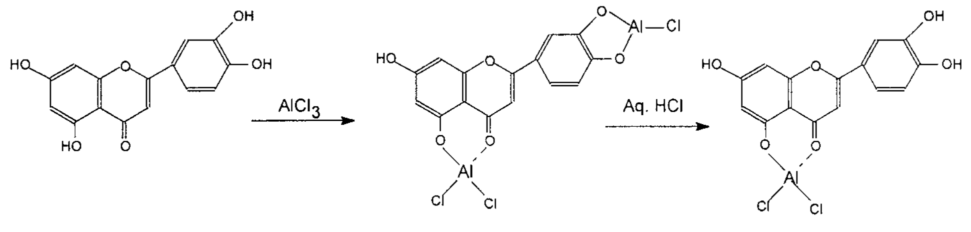



**Fig. (3)**

*$^{1}$H-NMR Spectral Analysis of Flavonoids* [4, p. 253-273]

For direct NMR analysis, highly methylated flavones and flavonols are soluble in $CDCl_3$, however, most flavonoids contain hydroxyl groups and/or they have sugar moieties, which are not soluble in $CDCl_3$. They are either dissolved in $CD_3OD$ or DMSO-$d_6$. Their methyl ethers or trimethylsilyl derivatives are prepared and sometimes their acetyl derivatives are used, in order to dissolve them in $CDCl_3$. When they are dissolved in DMSO-$d_6$, a small amount partially deuterated DMSO stays to give a narrow band between $\delta$ 2.4-2.6. Using DMSO-$d_6$ (if anhydrous) makes the observations of protons of hydroxyl groups possible such as $C_7$-OH at $\delta$ 10.93, $C_5$-OH at $\delta$ 12.40 and $C_3$-OH at $\delta$ 9.70. A trace amount of water broadens these peaks, thus the detection becomes difficult [4, p. 253-273]. Since the boiling point of DMSO-$d_6$ is $189^0$, the recovery of the flavonoid is quite impossible, also many of them undergo decomposition.

### *A-ring Protons*

In the A ring of flavones and flavonols at C-5 and C-7 positions have hydroxyl groups, thus C-6 and C-8 have protons. These protons are observed in the range δ 6.0-6.5 as doublets (J=2.5 Hz). H-6 doublet occurs consistently at higher field then the signal H-8. When a sugar is attached to the oxygen at C-7 the signals for both H-8 and H-6 are shifted down field (Table 1).

**Table 1. Chemical shift of C-6 and C-8 protons in flavones**

| | H-6 (δ, ppm) | H-8 (δ, ppm) |
|---|---|---|
| 5,7-dihydroxy flavon | 6.0-6.2 (d, J=2.5) | 6.3-6.5 (d, J=2.5) |
| 7-glycosyl | 6.2-6.4 | 6.5-6.9 |

The only other proton gives a signal in the same region is C-3 proton, which appears as a singlet near δ 6.3. Occasionally C-3' and C-5' proton signals will also appear in the same region of the spectrum. A useful technique to distinguish the C-6, C-8 and C-3 protons is to compare the NMR spectrum of fully trimethylsilylated compound with that of the compound containing trimethylsilyl substituents on all hydroxyl groups but the one at C-5. In the latter case, C-3 proton singlet is shifted downfield while C-8 proton signal is shifted upfield by about 0.15 ppm, while C-6 proton signal is almost uneffected.

### *B-ring Protons*

The protons in the B-ring usually appear in the range δ 6.7-7.9 which is downfield from the region where A-ring protons absorb. The protons have characteristic signals for the substitution pattern. Where there is C-4 oxygen substituent a typical four peak pattern of two doublets at δ 6.5-7.1 with J = 8.5 Hz is observed. Since the doublets for the C-3' and C-5' protons are shielded by the C-4' oxygen substituent always appears up-field from the C-2' and C-6' protons (7.1-7.3 ppm). In case 3', 4'-

dioxygenated flavones the B-ring spectrum is more complex than for the compounds with the 4-oxygenation (Table 2) shows 2', 5' and 6' protons.

**Table 2. Chemical shift of C-2', C-5' and C-6' protons**

| | H-2' | H-5' | H-6' |
|---|---|---|---|
| 5,7,3', 4'-tetra OH flavone | 7.2-7.3 | 6.7-7.1 | 7.3-7.5 |
| 3,5,7,3', 4'-penta OH flavone | 7.5-7.7 | 6.7-7.1 | 7.6-7.9 |
| 3,5,7,4'-tetra OH- 3'-OMe | 7.2-7.5 | 6.7-7.1 | 7.3-7.7 |

*C-ring Protons*

In flavones there is only C-3 proton, which gives a sharp singlet near δ 6.3. In flavonols this peak is not present.

*Sugar Protons*

The chemical shift of C-1'' proton of the sugar directly attached to the flavonoid hydroxyl group depends both on the nature of the flavonoid and on the position and stereochemistry of attachment to it. With a sugar (glucose etc.) on the C-3 hydroxyl the C-1'' proton signal is about δ 5.8, while the sugar at C-4', C-5 or C-7, C-1'' proton signal occurs much up field area near δ 5.0. Glucose commonly forms $\beta$-linkage in flavonoid glycosides, this $\beta$-linked sugar has a diaxial coupling with C-2'' proton, thus appears as a doublet with a coupling constant of about 7 Hz. In C-7 glucoside, however the C-1'' proton does not appear as a sharp doublet but gives a complex multiplet. Molecular models indicate a rotation at C-7 position, therefore C-1'' proton experiences different electronic environments. If there are substituents at C-6 and/or C-8, then the rotation is restricted and the expected doublet is observed. Rhamnose occurs as $\alpha$-L-rhamnoside, therefore C-1'' proton has an equatorial-equatorial coupling with C-2'' (J = 2 Hz) in both C-3 and C-7 rhamnosides the signal of C-1'' proton being at δ 5.0-5.3 (Table 3). Other sugar protons are observed between δ 3.0-4.0.

**Table 3.** C-1'' protons of glycosyl and rhamnosyl

| | Sugar C-1'' proton (δ, ppm) |
|---|---|
| 7-O-Glucoside | 4.8-5.2 |
| 3-O-Glucoside | 5.7-6.0 |
| 7-O-Rhamnoside | 5.1-5.3 |
| 3-O-Rhamnoside | 5.0-5.1 |

On the other hand, rhamnose methyl signal is observed as a broad peak or as a doublet at δ 0.7-1.0. Methoxyl protons of flavonoids usually appear in the region δ 3.5-4.1, and acetoxyl proton signals occur in the range δ 2.25-2.50.

### *$^{13}C$ NMR Analysis of Flavonoids*

$^{13}C$ NMR spectra of flavonoids are complementary to $^{1}H$ NMR spectra. Usually in $^{13}C$ NMR spectra some more compound is needed compared to $^{1}H$ NMR spectra. The same solvents such as $CDCl_3$, $CD_3OD$, DMSO-$d_6$, $C_6D_6$, and $C_5D_5N$ are used in both systems [5]. The chemical shift of some flavones and flavonols are given in Table 4.

### *Flavonoid O-glycosides*

Since sugar carbon resonances occur quite a different region than that of flavonoid nucleus, $^{13}C$ NMR gives good evidence to study the sugar moieties of the flavonoid glycosides. The sugars occurring in glycosidic form are distinguishable from one other by $^{13}C$ NMR spectroscopy. By this method C-1 configuration of sugars are identified as seen in Table 5. If one wants to record a spectrum in $CDCl_3$, it is better to prepare the acetyl derivatives of the glycosides. In such cases the acetylated carbon shifts downfield while adjacent carbon shifts upfield. The methyl signal of the acetyl group appears at δ 21.0 and the carbonyl at δ 171.0. Aromatic methoxyl groups are found about δ 55.0.

## *Mass Spectral Analysis of Flavonoids*

In order to record the mass spectra of flavonoids their permethyl or perdeutoromethyl derivatives are prepared, unless they are highly methylated [6]. In the EIMS spectrum usually $M^+$ is obtained, $[M\text{-}CO]^+$, $A_1^+$, $[A_1\text{-}CO]^+$, $[A_1\text{-}Me]^+$, $B_1$ and so on peaks are obtained. Figure 4 shows the degradation pattern of the flavonoids.

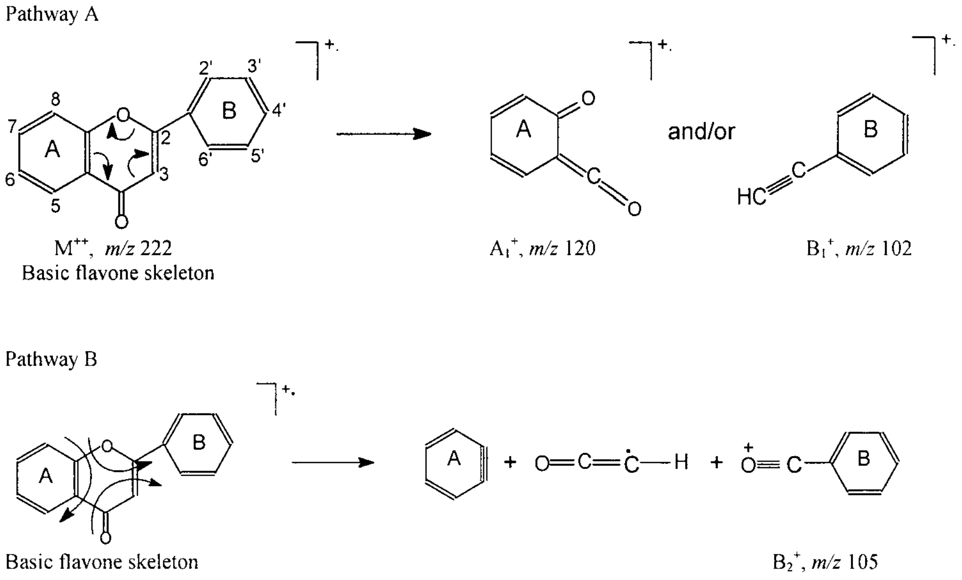



**Fig. (4)**

## *Genera in Turkish Labiatae Family*

Family Labiatae (Lamiaceae) consists of aromatic herbs or shrubs which are widely distributed around the Mediterranean coast and many other parts of the world . There are about 200 genera in Labiatae family. Since the absence of a satisfactory intra-familial classification, the genera are arranged in the following informal sequence in Turkey [7].

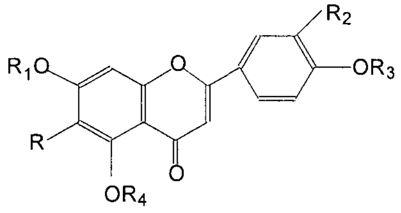
Many of these genera are used as folk medicine throughout the world. Among them *Salvia* is one of the most studied genus.This article therefore will start with the flavonoids of *Salvia*.

| | | |
|---|---|---|
| 1-Ajuga | 16-Ballota | 31-Calamintha |
| 2-Teucrium | 17-Marrubium | 32-Clinopodium |
| 3-Rosmarinus | 18-Sideritis | 33-Acinos |
| 4-Lavandula | 19-Stachys | 34-Micromeria |
| 5-Prosium | 20-Melissa | 35-Cyclotrichium |
| 6-Scutellaria | 21-Nepeta | 36-Thymus |
| 7-Melittis | 22-Glechoma | 37-Coridothymus |
| 8-Eremostachys | 23-Dracocephalum | 38-Thymbra |
| 9-Phlomis | 24-Lallemantia | 39-Mentha |
| 10-Lamium | 25-Hymenocrater | 40-Lycopus |
| 11-Wiedemannia | 26-Hyssopus | 41-Ziziphora |
| 12-Galeobdolon | 27-Prunella | 42-Salvia |
| 13-Galeopsis | 28-Origanum | 43-Drystoechas |
| 14-Leonurus | 29-Pentapleura | 44-Elsholtzia |
| 15-Moluccella | 30-Satujera | 45-Ocimum |

## *SALVIA* FLAVONOIDS

In our first study with *Salvia* species, *S. triloba* L.f. [8] we have isolated a new flavone salvigenin (1) (6,7,4'-trimethylapigenin), later the same compound was obtained from other *Salvia* species. From *S. tomentosa* [9] cirsimaritin (**2**), jaceosidin (**3**), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**4**), 6-methoxyluteolin (**5**), luteolin (**6**), luteolin 7-glucoside (**7**) were isolated, in an other study with the same plant [10] further compounds eupatilin (**8**), cirsilineol (**9**), diosmetin (**10**), 6-hydroxyluteolin 7- and 5-glucosides (**11** and **12**) were obtained.
5-Methoxysalvigenin (**13**) was obtained from *S. officinalis* [11]. From *Salvia palaestina* Bentham [12] sixteen flavonoids, ten aglycones and six glycosides were isolated, these were salvigenin (**1**), eupatilin (**8**), luteolin 7,4'-dimethyl ether (**15**), genkwanin (**16**), cirsimaritin (**2**), chrysoeriol (**17**), apigenin (**18**), luteolin (**6**), apigenin 7-glucoside (**19**), apigenin 7-glucuronide (**20**), luteolin 7-glucoside (**7**), luteolin 7-glucuronide (**21**), chrysoeriol 7-glucoside (**22**), chrysoeriol 7- glucuronide (**23**). From *Salvia*

*verticillata* [13] a rare flavone glycoside 6-hydroxyluteolin 5-O-glucoside (**12**) was obtained which was also found in *S. tomentosa* [10] previously.

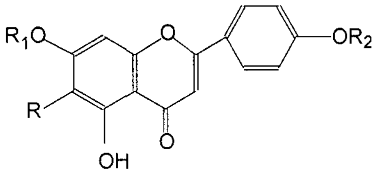



| | R | R1 | R2 | R3 | R4 |
|---|---|---|---|---|---|
| **1** | OMe | Me | H | Me | H |
| **2** | OMe | Me | H | H | H |
| **3** | OMe | H | OMe | H | H |
| **4** | OMe | Me | OMe | Me | H |
| **5** | OMe | H | OH | H | H |
| **6** | H | H | OH | H | H |
| **7** | H | Glc | OH | H | H |
| **8** | OMe | H | OMe | Me | H |
| **9** | OMe | Me | OMe | H | H |
| **10** | H | H | OMe | H | H |
| **11** | OH | Glc | OH | H | H |
| **12** | OH | H | OH | H | Glc |
| **13** | OMe | Me | H | Me | Me |

Glc = Glucose

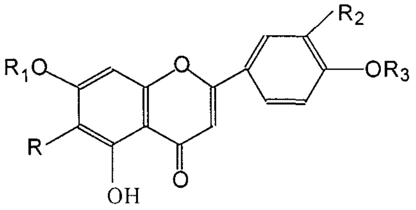
Same plant has also yielded cirsimaritin (**2**) and luteolin 7-glucoside (**7**). *Salvia pinnata* [13] has yielded apigenin (**18**), luteolin (**6**), 6-methoxyluteolin (**5**), apigenin 7-O-glucoside (**19**) and quercetin 3-*O*-glucoside (**24**). From *S. sclarea* [14] apigenin (**18**), luteolin (**6**), 4'-methyl apigenin (**25**) and 5-hydroxy-6,7,3',4'-tetramethylflavone (**4**), 6-hydroxyapigenin-7,4'-dimethyl ether (**26**), and from *S. nemorosa* [15] four flavone aglycones, namely salvigenin (**1**), eupatilin (**8**), apigenin (**18**) and luteolin (**6**) were isolated. *S. euphratica* [16] has yielded 7,3',4'-trimethoxy luteolin (**27**) and luteolin 7-glucoside (**7**). On the other hand, *S. longipedicellata* [16] consist of flavonols quercetin 3-methyl ether (**28**), quercetin-3,3'-dimethyl ether (**29**), 6-hydroxykaempferol-3,6,4'-trimethyl ether (**30**), 6-hydroxykaempferol-3,6-dimethyl ether (**31**) and kaempferol-3,7-dimethyl ether (**32**) which also found in *S. cyanescens* [17], this latter

plant further yielded salvigenin (**1**) and 6-hydroxy-7,4'-dimethoxy-apigenin (**33**).

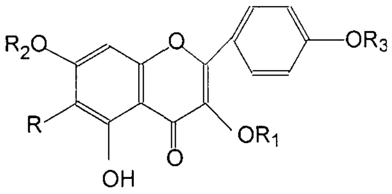



| | R | R1 | R2 |
|---|---|---|---|
| **14** | OMe | H | Me |
| **16** | H | Me | H |
| **18** | H | H | H |
| **19** | H | Glc | H |
| **20** | H | Glucu | H |
| **25** | H | H | Me |
| **33** | OH | Me | Me |
| **40** | H | Glc-Glc | H |
| **41** | H | Glc-Glc | Glc |
| **42** | OMe | H | H |
| **45** | OMe | Glucu | H |
| **46** | OMe | Glc | H |
| **48** | OH | Me | H |

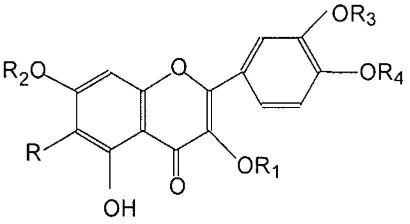
Glucu = Glucuronide




| | R | R1 | R2 | R3 |
|---|---|---|---|---|
| **15** | H | Me | OH | Me |
| **17** | H | H | OMe | H |
| **21** | H | Glucu | H | H |
| **22** | H | Glc | OMe | H |
| **23** | H | Glucu | OMe | H |
| **26** | OH | Me | H | Me |
| **27** | H | Me | OMe | Me |
| **34** | OH | Me | OH | H |
| **43** | H | H | Glucu | H |
| **47** | OMe | Glc | OH | H |
| **49** | OMe | Me | OH | Me |

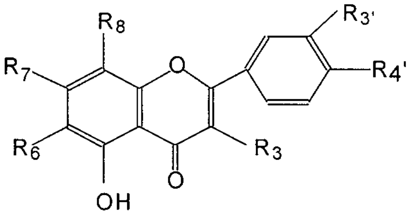



| | R | R1 | R2 | R3 |
|---|---|---|---|---|
| **30** | Me | OMe | H | Me |
| **31** | OMe | Me | H | H |
| **32** | H | Me | Me | H |




| | R | R1 | R2 | R3 | R4 |
|---|---|---|---|---|---|
| **24** | H | Glc | H | H | H |
| **28** | H | Me | H | H | H |
| **29** | H | Me | H | Me | H |

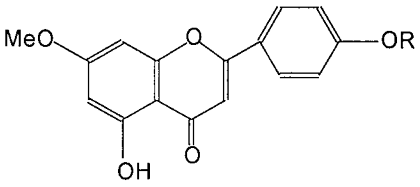
*S. blepharophylla* Brandegee ex. Epling [18] has yielded eight flavone and flavone glycosides such as pedalitin (**34**), diosmetin (**10**), quercetin 3-*O*-glucoside (**24**), vitexin (**35**), schaftosid (**36**), and miquelianin (**37**) (quercetin 3-O-$\beta$-D-glucuronopyranoside).

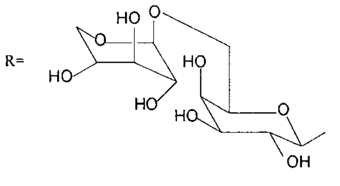


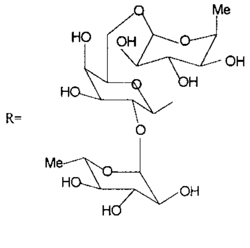

| | $R_3$ | $R_6$ | $R_7$ | $R_8$ | $R_3$' | $R_4$' |
|---|---|---|---|---|---|---|
| **35** | H | H | OH | C- Glc | H | OH |
| **36** | H | C- Glc | OH | C-Ara | H | OH |
| **37** | O-Glucu | H | OH | H | OH | OH |
| **44** | H | C- Glc | OH | C- Glc | H | OH |

Ara = Arabinose

*Salvia moorcroftiana* Wall. has yielded two new flavone glycosides genkwanin 4'-*O*-$\alpha$-L-arabinopyranosyl (1→6)-$\beta$-D-galactopyranoside (**38**) and genkwanin 4'-*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-[$\alpha$-L-rhamno-pyranosyl-(1→6)- $\beta$-D-galactopyranoside (**39**) [19].



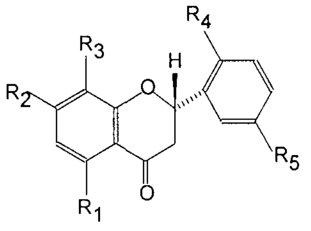



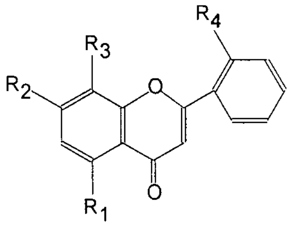

**38** **39**

Two new flavone glycosides with an unusual linkage have been isolated from the interglycosidic petals of *S. uliginosa* [20] and identified as apigenin 7-*O*-$\beta$-D-glucopyranosyl-(1'''→4'')-$\beta$-D-glucopyranoside (**40**) and apigenin 7-*O*-$\beta$-D-glucopyranosyl-(1'''→4'')-$\beta$-D-glucopyranoside-4'-*O*-$\beta$-D-glucopyranoside (**41**). Using a reversed-phase Spherisorb $ODS_2$ column, six phenolic compounds were separated from sage (*S. officinalis*) [21] three of them were flavonoids, luteolin 7-glucoside (**7**), apigenin (**18**), and hispidulin (**42**). Luteolin 7-O-$\beta$-D-glucoside (**7**), 7-*O*-$\beta$-glucuronide (**21**), 3'-*O*-$\beta$-glucuronide (**43**), 6-hydroxyluteolin 7-*O*-$\beta$-D-glucoside (**11**), vicenin-2 (**44**) (6,8-di-C-$\beta$-glucosylapigenin) were isolated from sage, this is the first finding of a C-glycoside in *S. officinalis* [22].
From *Salvia plebeia* [23] hispidulin 7-glucuronide (**45**), hispidulin 7-*O*-D-glucoside (**46**), and 6-methoxyluteolin 7-glucoside (**47**) were isolated, the compounds, especially **47** showed strong antioxidant activity. *Salvia moorcroftiana* [24] has yielded four known flavonoids apigenin (**18**), sorbifolin (**48**), pectolinarigenin (**14**) and eupatorin (**49**).

## *SCUTELLARIA* FLAVONOIDS

Some cytotoxic flavonoids were isolated from *Scutellaria indica* [25] these were; 2(S)-5,7-dihydroxy-8,2'-dimethoxyflavanone (**50**), 2(S)-5,7,2'-trihydroxy-8-methoxyflavanone (**51**), 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone (**52**) and wogonin (5,7-dihydroxy-8-methoxy-flavone) (**53**), 5,7-dihydroxy-8,2'-dimethoxyflavone (**54**).

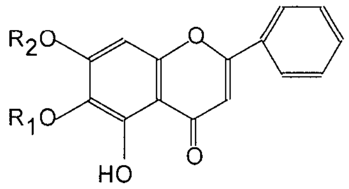


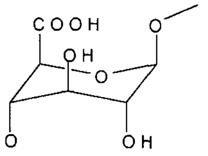

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **50** | OH | OH | OMe | OMe | H |
| **51** | OH | OH | OMe | OH | H |
| **52** | OH | OMe | OMe | OH | OH |

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **53** | OH | OH | OMe | H |
| **54** | OH | OH | OMe | OMe |

*S. baicalensis* has yielded wogonin (**53**), baicalein (**55**) and baicalin (**56**). These flavonoids have both antiinflammatory effect and cause cellular activation, enhanced the synthesis of both collagen and total protein [26].

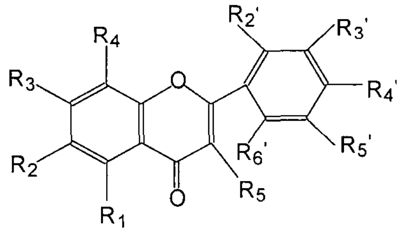



**55** $R_1 = H$ $R_2 = H$

**56** $R_1 = H$ $R_2 =$ 


**57** $R_1 = Me$ $R_2 = H$

From the roots of the same plant in addition to baicalein (**55**), oroxylin A (**57**) and skullcapflavon II (**58**) were isolated and their binding activities were tested [27]. *S. planipes* has yielded three new and ten known flavonoids 5,7,3',6'-tetrahydroxy-6,8,2'-trimethoxyflavone (**59**), 7,2'-dihydroxy-5-methoxyflavone (**60**), (2R,3R)-3,5,7,2',5'pentahydroxyflavanone (**61**) together with known flavones skullcapflavone I (**62**), wogonin (**53**), baicalein (**55**), norwogonin (**63**), viscidulin III (**64**), 5,7,2',6'-tetrahydroxyflavone (**65**), 3,5,7,2',6'-pentahydroxyflavone (**66**), wogonin 7-*O*-*β*-D-glucuronoide (**67**), baicalin (**56**), baicalein 7-*O*-*β*-D-glucopyranoside (**68**) [28]. *Scutellaria ocellata* and *S. nepetoides* have yielded known flavonoids oroxylin A (**57**), wogonin (**53**), apigenin (**18**), 3,7,4'-trihydroxyflavone (**69**), cinaroside (**7**), baicalin (**56**) and wogonoside (**70**) as well as apigenin 7-O-*β*-D-glucuronoide (**20**), norwogonoside (**71**), scutellarein (**72**), a new flavone glycoside nepetoside A (5,8-dihydroxy 7-*O*-*β*-D-galacturonidopyranosylflavone) (**73**) [29]. Aerial parts of *S. immaculate* has yielded a group of flavone glycosides chrysin 7-*O*-glucuronide (**74**), scutellarein 7-*O*-glucoside (**75**), apigenin 7-

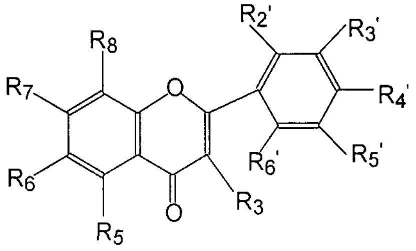



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_2$' | $R_3$' | $R_4$' | $R_5$' | $R_6$' |
|---|---|---|---|---|---|---|---|---|---|---|
| **58** | OH | OMe | OMe | OMe | H | OH | H | H | H | OMe |
| **59** | OH | OMe | OH | OMe | H | OMe | OH | H | H | OH |
| **60** | OMe | H | OH | H | H | OH | H | H | H | H |
| **62** | OH | H | OMe | OMe | H | OH | H | H | H | H |
| **63** | OH | H | OH | OH | H | H | H | H | H | H |
| **64** | OH | H | OH | OMe | H | OH | H | H | OH | OMe |
| **64a** | OH | H | OH | OMe | H | OH | H | H | H | OMe |
| **65** | OH | H | OH | H | H | OH | H | H | H | OH |
| **66** | OH | H | OH | H | OH | OH | H | H | H | OH |
| **67** | OH | H | O-Glucu | OMe | H | H | H | H | H | H |
| **68** | OH | OH | O-Glc | H | H | H | H | H | H | H |

*O*-glucoside (**19**), baicalein 7-*O*-glucoside (**76**), norwogonin 7-*O*-glucoside (**72**) and a new glycoside immaculoside (**77**) (5,8-dimethoxy 7-*O*-$\beta$-D-glucopyranosylflavone) [30]. From the aerial parts of *S. pontica* isovitexin (**78**), apigenin (**18**), apigenin 7-*O*-$\beta$-D-glucopyranoside (**19**), apigenin-4'-*O*-methyl ether 7-*O*-$\beta$-D-glucopyranoside (**79**), 5-hydroxy-7,3',4'-trimethoxyflavone (**27**) were isolated [31].

In two studies with the aerial parts of *Scutellaria przewalskii* apigenin (**18**), luteolin (**6**), scutellarein (**72**), dinatin (**42**), pectolinarigenin (**14**), hispidulin (**42**), apigenin 7-glucoside (**19**), luteolin 7-glucoside (**7**), scutellarein 7-glucoside (**75**), apigenin 7-glucuronide (**20**), dinatin 7-glucuronide (**45**), and their glucobiosides were isolated [32]. In a second study with the same plant baicalin (**56**) was obtained [33]. *S. baicalensis* has yielded six new flavonoids; 5,7,2'-trihydroxy-8-methoxyflavone (**80**), 5,2',6'-trihydroxy-7,8-dimethoxyflavone (**81**), 5,7,2'-trihydroxy-6'-methoxyflavone (**82**), 5,7,2',3'-tetrahydroxyflavone (**83**), 3,5,7,2',6'-pentahydroxyflavone (**84**), 2(S)-7,2',6'-trihydroxy-5-methoxyflavanone

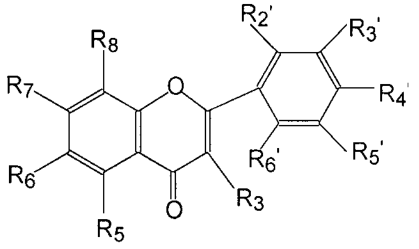
(**85**), 2,6,2',4'-tetrahydroxy-6'-methoxychalcone (**86**) [34]. Later the same plant has yielded three more new compounds 5,7,2',5'-tetrahydroxy-8,6'-dimethoxyflavone (**64**), 5,7,2'-trihydroxy-8,6'-dimethoxyflavone (**64a**), 5,2',5'-trihydroxy-6,7,8-trimethoxyflavone (**87**) [35].




| | $R_3$ | $R_5$ | $R_6$ | $R_7$ | $R_8$ | $R_{2'}$ | $R_{3'}$ | $R_{4'}$ | $R_{5'}$ | $R_{6'}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **69** | OH | H | H | OH | H | H | H | OH | H | H |
| **70** | H | OH | H | O-Glucu | OMe | H | H | H | H | H |
| **71** | H | OH | H | O-Glc | OH | H | H | H | H | H |
| **72** | H | OH | OH | OH | H | H | H | OH | H | H |
| **73** | H | OH | H | O-Galac | OH | H | H | H | H | H |
| **74** | H | OH | H | O-Glucu | OH | H | H | H | H | H |
| **75** | H | OH | OH | Glc | H | H | H | OH | H | H |
| **76** | H | OH | OH | Glc | H | H | H | H | H | H |
| **77** | H | OMe | H | Glc | OMe | H | H | H | H | H |
| **78** | H | OH | C-Glc | OH | H | H | H | OH | H | H |
| **79** | H | OH | H | O-Glc | H | H | H | OMe | H | H |

Galac = Galacturonide

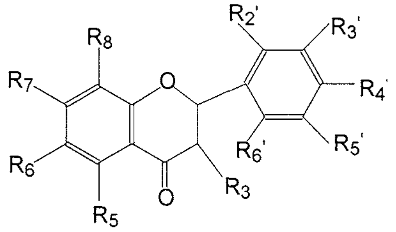
*S. altissima* has yielded one new and six known compounds baicalein (**55**), baicalin (**56**), wogonin (**53**), oroxylin (**57**), wogonoside (**70**), oroxyloside (**88**), and the new compound altisin (**89**) [36]. From *S. ovata* seven flavones were obtained, chrysin (**90**), apigenin (**18**), luteolin (**6**), 6-methoxyluteolin (**5**), dinatin (**42**) and two new compounds oroxylin 7-O-glucoside (**91**) and ovatin (**92**) [37]. Two new C-glycosides were isolated from *S. baicalensis* the compounds were chrysin 6-C-β-D-glucoside-8-C-α-L-arabinoside (**93**) and chrysin 6-C-α-L-arabinoside-8-C-β-D-glucoside (**94**) [38].




| | $R_3$ | $R_5$ | $R_6$ | $R_7$ | $R_8$ | $R_{2'}$ | $R_{3'}$ | $R_{4'}$ | $R_{5'}$ | $R_{6'}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **80** | H | OH | H | OH | OMe | OH | H | H | H | H |
| **81** | H | OH | H | OMe | OMe | OH | H | H | H | OH |
| **82** | H | OH | H | OH | H | OH | H | H | H | OMe |
| **83** | H | OH | H | OH | H | OH | OH | H | H | H |
| **84** | OH | OH | H | OH | H | OH | H | H | H | OH |
| **87** | H | OH | OMe | OMe | OMe | OH | H | H | OH | H |
| **88** | H | OH | OMe | O-Xyl | H | H | H | H | H | H |
| **89** | H | OH | H | OMe | OMe | OMe | H | H | H | OMe |
| **90** | H | OH | H | OH | H | H | H | H | H | H |
| **91** | H | OH | OMe | O-Glc | H | H | H | H | H | H |
| **92** | H | OMe | OMe | O-Glc | H | H | H | H | H | H |
| **93** | H | OH | C-Glc | OH | C-Ara | H | H | H | H | H |
| **94** | H | OH | C-Ara | OH | C-Glc | H | H | H | H | H |

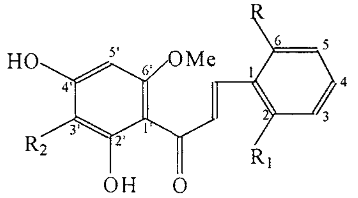
Xyl = Xyloside

Four flavanones and a new chalcone were isolated from the roots of *Scutellaria discolor* together with wogonin (**53**), norwogonin (**63**), 5,7,2'-trihydroxy-8-methoxyflavone (**80**), 5,7-dihydroxy-8,2'-dimethoxyflavone (**54**), wogonin 7-O-glucuronide (**70**). The new flavanones 2(S)-5,7-dihydroxy-8,2'-dimethoxyflavanone (**50**), (+) - 5,2'-dihydroxy-7,8,6'-tri-methoxyflavanone (**95**), (+)-5,2'-dihydroxy-6,7,6'-trimethoxyflavanone (**96**), 2',4'-dihydroxy-2,3',6'-trimethoxychalcone (**97**) [39]. *S. indica* has also yielded flavanones 2(S)-5,7,2'-trihydroxy-8-methoxyflavanone (**98**), 2(S)-5,7,2'-trihydroxyflavanone (**99**), 2(S)-5,2',5'-trihydroxy-7,8-dimethoxyflavanone (**52**), 2(S)-5,2'-dihydroxy-7,8,6'-trimethoxyflavanone 2'-O-β-glucuronopyranoside (**100**) as well as known flavones [40].



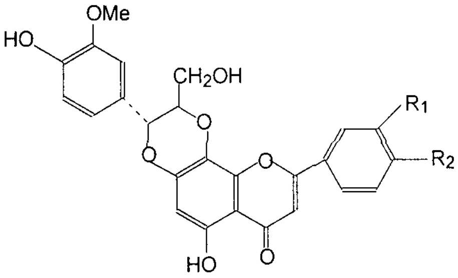

| | $R_3$ | $R_5$ | $R_6$ | $R_7$ | $R_8$ | $R_{2'}$ | $R_{3'}$ | $R_{4'}$ | $R_{5'}$ | $R_{6'}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **61** | OH | OH | H | OH | H | OH | H | H | OH | H |
| **85** | H | OMe | H | OH | H | OH | H | H | H | OH |
| **95** | H | OH | H | OMe | OMe | OH | H | H | H | OMe |
| **96** | H | OH | OMe | OMe | H | OH | H | H | H | OMe |
| **98** | H | OH | H | OH | OMe | OH | H | H | H | H |
| **99** | H | OH | H | OH | H | OH | H | H | H | H |
| **100** | H | OH | H | OMe | OMe | OGlucu | H | H | H | OMe |
| **101** | H | OH | OMe | OH | H | OH | H | H | OH | H |
| **110** | OMe | OMe | H | OMe | H | OMe | H | H | H | OMe |
| **112** | H | OH | H | OH | H | H | OH | OH | H | H |
| **152** | H | OH | OMe | OMe | H | H | OMe | OMe | H | H |
| **158** | H | OH | H | ORut | H | H | OH | OMe | H | H |
| **175** | H | OH | H | OH | H | H | H | OH | H | H |
| **186** | H | OH | H | OGlc | H | H | H | OH | H | H |
| **187** | H | OH | H | OCouGlc | H | H | H | OH | H | H |

Rut = Rutinoside
CouGlc = Coumaroylglucoside

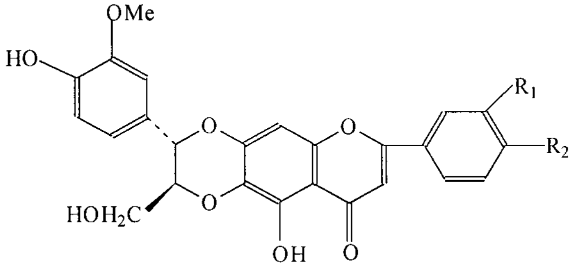



| | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **86** | OH | OH | H |
| **97** | H | OMe | OMe |

*S. scandens* has yielded five flavanones, four of them are known compounds with acylglycosylation. The new compound **101** has substitution at 2' position, 5,7,2',5'-tetrahydroxy-6-methoxyflavanone [41]. From the roots of *S. prostrata* interesting flavonoids scutellaprostins A (**102**), B (**103**), C (**104**), D (**105**), E (**106**), and F (**107**) were isolated. Their structures were established also by synthesis [42].

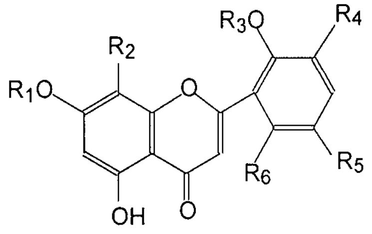



| | $R_1$ | $R_2$ |
|---|---|---|
| **102** | H | H |
| **103** | H | OH |
| **104** | OH | OH |



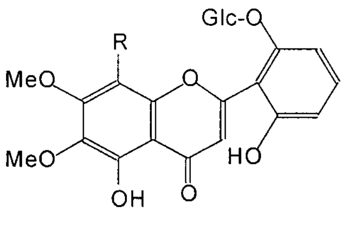

| | $R_1$ | $R_2$ |
|---|---|---|
| **105** | H | H |
| **106** | H | OH |
| **107** | OH | OH |

*S. baicalensis* is used against various sicknesses in China and Japan. A number of flavonoids have been isolated from this plant, including viscidulin III (**64**), oroxylin A (**57**), wogonin (**53**), baicalein (**55**),viscidulin II (**108**), scullcapflavone II (**62**), chrysin (**90**), 5,7,2'-trihydroxy-8,6'-dimethoxyflavone (**109**) and (2R, 3R) - 3,5,7,2',6'-pentamethoxyflavanone (**110**) and a new flavone viscidulin III 2'-O-β-D-glucopyranoside (**111**) as well as eriodictyol (**112**) and rivularin (**113**) [43].



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|---|
| **109** | H | OMe | H | H | H | OMe |
| **111** | H | OMe | Me | OH | H | O-Glc |
| **113** | OMe | OMe | H | H | H | OMe |

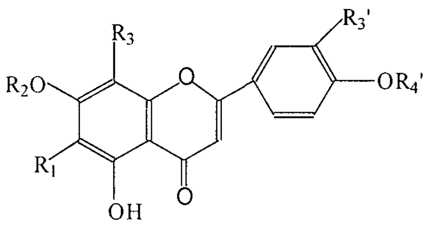
Two new flavonoid glycosides as 2' glycosides were found in *S. baicalensis* 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone 2'-O-glucoside (**114**), 5,2',6'-trihydroxy-6,7-dimethoxyflavone 2'-O-glucoside (**115**) [44].



**114** **R** = OMe
**115** **R** = H

## *TEUCRIUM* FLAVONOIDS

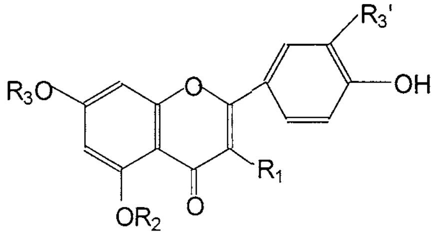
The first flavonoid study with *Teucrium fruticans* L. [45] was carried out recently and the known flavonoids were isolated 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**4**), cirsilineol (**9**) and predominantly cirsimaritin (**2**). The flavonoids obtained from two *Teucrium* species namely *T. leucocladum* and *T. polium* were discussed for the chemosystematic relationship [46], the flavonoids were apigenin 7-glucoside (**19**), vicenin-2 (**44**), luteolin 7-glucoside (**7**) and cirsimaritin (**2**). A chemotaxonomic study of the flavonoids of *Teucrium* species was carried out by Harborne et al. [47]. In this survey with the aerial parts of 42 European taxa of genus *Teucrium* it was found that five flavonoids are wide spread, these were cirsiliol (**116**), cirsimaritin (**2**), cirsilineol (**9**), salvigenin (**1**) and 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**4**). Compounds **1** and **4** are found to be useful as taxonomic markers. Salvigenin (**1**) is characteristic of species of section *Polium* while (**4**) is completely confined to species of the other five sections surveyed. Eleven flavone glycosides, four flavonol glycosides and C-flavones vicenin-2 (**44**) were found to occur as vacuolar constituents. One of the flavone glycoside cirsimaritin 4'-glucoside (**117**), only occurs in *T. arduini*, while two others hypolaetin (**118**) and isoscutellarein 7-acetylallosyl glucoside (**119**) are found in closely related *T. chamaedrys* and *T. webbianum*. 6-Hydroxyluteolin is widely present as the 7-glucoside (**11**) and 7-rhamnoside (**120**), the latter being a new glycoside.




| | $R_1$ | $R_2$ | $R_3$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|
| **116** | OMe | Me | H | OH | OH |
| **117** | OMe | Me | H | H | Glc |
| **118** | H | H | OH | OH | H |
| **120** | OH | Rha | H | OH | OH |

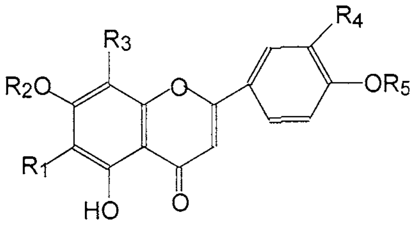
# *OCIMUM FLAVONOIDS*

In a comprehensive study on the chemosystematic characters of genus *Ocimum* [48] nine species belonging to three subgenera of *Ocimum* were investigated, among them only *O. americanum* var. *pilosum* has C-glycosides, vicenin-2 (**44**), this compound is present in var. *americanum* only in trace amount. The major flavonoids in all subspecies of *Ocimum* were flavonol 3-glucosides and 3-rutinosides, also an unusual compound quercetin 3-O-(6''-O-malonyl)glucoside (**121**) was obtained. The compounds were identified by UV and NMR spectroscopy after separation by 2D-paper chromatography and semipreparative HPLC in above given study. From *O. americanum* var. *pilosum* vicenin 2 (**44**), *O. kilimandscharicum* L. rutin (**122**), galuteolin (luteolin 5-O-glucoside) (**123**), and from *O. tenuiflorum* apigenin 7-O-glucuronide (**20**) and luteolin 7-O-glucuronide (**21**), from *O. lamiifolium* quercetin 3-O-xylosyl-(1'''→ 2'') galactoside (**124**), from *O. minimum* isoquercitrin (**24**) and compound **121** as well as six more flavone glycosides quercetin 3-O-xylosyl-(1'''→2'')-galactoside (**124**), luteolin 7-glucoside (**7**), luteolin 5-glucoside (**123**), apigenin 5-glucoside (**125**), apigenin 7-glucoside (**19**), kaempferol 3-rhamno-(1'''→6'')-glucoside (**126**), kaempferol 3-glucoside (**127**) and kaempferol 3-O-malonylglucoside (**128**) were isolated. 8-Oxygenated flavonoids were also obtained from genus *Ocimum* [49]. *O. canum* Sims (= *O. americanum*) has yielded nevadensin (**129**), salvigenin (**1**) and hispidulin (**42**), together with minor amounts of cirsiliol (**116**), cirsilineol (**9**), eupatorin (**49**), apigenin (**18**), acacetin (**25**), genkwanin (**16**), cirsimaritin (**2**), ladanein (**130**) and gardenin B (**131**) [50]. The presence of xanthomicrol (**132**) and isothymusin (**133**) were reported in *O. grandiflorum* Lam. [51]. 8-Hydroxyflavones could easily be distinguished in UV spectra. The most characteristic feature being a strong Band III at around 300 nm, but 8-hydroxylated compounds are unstable at room temperature. Sixteen accessions of *O. basilicum* L. belonging to different cultivars were examined for the external leaf flavonoids and one or two major flavone aglycones such as salvigenin (**1**) and nevadensin (**129**) were found. About ten minor flavonoids were established cirsiliol (**116**), cirsilineol (**9**), eupatorin (**49**), apigenin (**18**), acacetin (**25**), genkwanin (**16**), apigenin-7,4'-dimethyl ether (**134**), cirsimaritin (**2**), ladanein (**130**) and gardenin B (**131**) [52]. An Indian plant *O. sanctum* has yielded two flavonoids orientin (**135**) and vicenin-2 (**44**) [53].




| | $R_1$ | $R_2$ | $R_3$ | $R_{3'}$ |
|---|---|---|---|---|
| **121** | O-(6''-O-Mal)Glc | H | H | OH |
| **122** | O-Rha(1'''→6'')Glc | H | H | OH |
| **123** | H | Glc | OH | OH |
| **124** | O-Xyl(1'''→2'')Gal | H | H | OH |
| **125** | H | Glc | H | H |
| **126** | O-Rha(1'''→6'')Glc | H | OH | H |
| **127** | O-Glc | H | OH | H |
| **128** | O-MalGlc | H | OH | H |

MalGlc = Malonylglucoside
RhaGlc = Rhamnosylglucoside
XylGal = Xylosylgalactoside

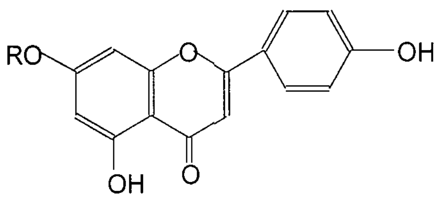



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **129** | OMe | H | OMe | H | Me |
| **130** | OH | Me | H | H | H |
| **131** | OMe | Me | OMe | H | Me |
| **132** | OMe | Me | OMe | H | H |
| **133** | OMe | Me | OH | H | H |
| **134** | H | Me | H | H | Me |
| **135** | H | H | C-Glc | OH | H |

Using liquid chromatography coupled to mass spectrometry, common flavonoids were detected from *O. gratissimum* var. *gratissimum*, the flavones were characterized as xanthomicrol (**132**), cirsimaritin (**2**), rutin (**122**), kaempferol 3-O-rutinoside (**126**) and vicenin-2 (**44**) as major flavonoids and luteolin 5-O-glucoside (**123**), luteolin 7-O-glucoside (**7**), apigenin 7-O-glucoside (**19**), vitexin (**35**), isovitexin (**78**), quercetin 3-O-glucoside (**24**) and isothymusin (**133**) were detected as minor flavonoids [54].

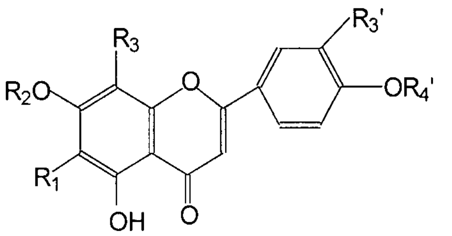
## SIDERITIS FLAVONOIDS

In Spanish *Sideritis* species mainly 8-hydroxyflavonoids were found. In *S. mugronensis, S. tragoriganum* and *S. reverchonii* hypolaetin 8-glucuronide (**136**), hypolaetin 4'-methyl ether 8-glucuronide (**137**), and hypolaetin 4'-methyl ether 8-glucoside (**138**) have been identified for the first time in Labiatae family [55]. Apigenin (**18**) and luteolin (**6**) were isolated from *S. raeseri* [56]. *S. hyssopifolia* has yielded a new glycoside together with three known flavone glycosides. The new compound was 4'-O-methylisoscutellarein 7-O-[6'''-O-acetyl-β-D-allopyranosyl-(1→2)]-6''-O-acetyl-β-D-glucopyranoside (**139**) [57]. Methanolic extract of *S. mugronensis* has yielded hypolaetin 8-O-glucoside (**140**) with antiinflammatory and antirheumatic activity [58], *S. leucantha* also yielded compound **140** [59]. Ethyl acetate extract of *S. luteola* Cav. worked out using HPLC, a new flavonoid isoscutellarein 7-O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside (**141**) was isolated, its structure was elucidated by $^1$H and $^{13}$C NMR spectra [60].




**143** R = 6''-O-p-coumaroylglucoside
**144** R = 4''-O-p-coumaroylglucoside

Three acylated flavone glycosides were isolated from *S. syriaca* L. [61], these were 5,8,3'-trihydroxy-4'-methoxyflavone 7-O-(6'''-O-acetylsophoroside) (**142**), apigenin 7-O-(6''-p-coumaroylglucoside) (**143**) and apigenin 7-O-(4''-p-coumaroylglucoside) (**144**).

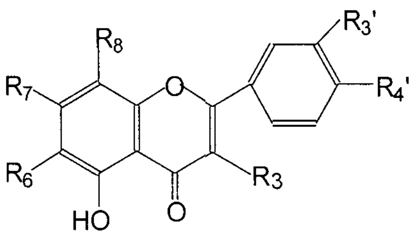


| | $R_1$ | $R_2$ | $R_3$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|
| **136** | H | H | Glucu | OH | H |
| **137** | H | H | Glucu | OH | Me |
| **138** | H | H | Glc | OH | Me |
| **139** | H | AcAllo-AcGlc | OH | H | Me |
| **140** | H | OH | O-Glc | OH | H |
| **141** | H | Glc-Xyl | OH | H | H |
| **142** | H | AcSoph | OH | OH | Me |
| **145** | OMe | Me | O-Glc | OH | H |
| **146** | H | AlloGlc | OH | OH | H |

AcGlc = Acetylglucopyranoside
AcAllo = Acetylallopyranoside
AcSoph = Acetylsophoroside
AlloGlc = Alloglucopyranoside

In several other *Sideritis* species p-coumaroylglucosides have been reported. Two new flavonoid glycosides 5,8,3',4'-tetrahydroxy-6,7-dimethoxyflavone 8-β-D-glucoside (**145**) and 8-hydroxyluteolin 7-(2''-O-allosylglucoside) (**146**) were obtained from *S. leucantha* for the first time in nature, allose is also a rare natural sugar [62]. Apigenin 7-glucoside (**19**) and compound **144** were obtained from *S. raeseri* [63]. Methoxylated

flavonoids were obtained from *S. mugronensis* [64], such as 5-O-desmethylnobiletin (**147**) and gardenin D (**148**). From *S. bolleana* Bornm. 3,7,4'-trimethoxykaempferol (**149**) and *S. gomerae* Bolle pectolinarigenin (**14**), artemetin (**150**), eupatorin (**49**) were obtained [65]. From *S. lotsy* (Pit.) Ceb. et Ort., *S. marmorea* Bolle and *S. nutans* Svent 3,7,4'-trimethoxykaempferol (**149**) was isolated, from the two latter species 5-hydroxy-7,4'-dimethoxyflavone (**134**) was also isolated, *S. nutans* contained 5-hydroxy-3,6,7,4'-tetramethoxyflavone (**151**), salvigenin (**1**), eupatorin (**49**), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**4**) [66]. From *S. cystosiphon* 5-hydroxy-6,7,3',4'-tetramethoxyflavanone (**152**) was isolated [66].




| | $R_3$ | $R_6$ | $R_7$ | $R_8$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|---|
| **147** | H | OMe | OMe | OMe | OMe | OMe |
| **148** | H | OMe | OMe | OMe | OH | OMe |
| **149** | OMe | H | OMe | H | H | OMe |
| **150** | OMe | OMe | OMe | H | OMe | OMe |
| **151** | OMe | OMe | OMe | H | H | OMe |
| **153** | H | OMe | OMe | OMe | OMe | H |
| **154** | H | OMe | OMe | OMe | OH | OH |
| **156** | H | OMe | O-Glc | OMe | OH | OH |
| **157** | H | OMe | O-Glc | OMe | OMe | OH |

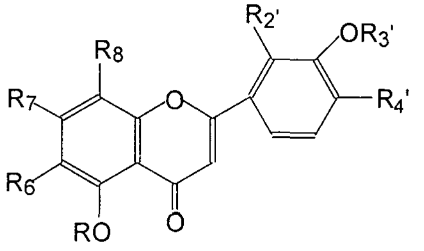
Antioxidant activity was established in some flavonoids of *S. javalambrensis* Pau which is an endemic Spanish plant [67]. From this species both aglycones cirsiliol (**116**), cirsimaritin (**2**), 8-methoxy-cirsilineol (**153**), sideritoflavone (**154**) and xanthomicrol (**132**), as well as gylcosides such as 4'-O-methylisoscutellarein 7-O-[6'''-acetylallopyranosyl(1→2) glucopyranoside] (**139**), its deacetyl derivative, isoscutella-rein 7-O-[6'''-acetyl-allopyranosyl(1→2)glucopyranoside] (**119**), 3'-

hydroxy-4'-O-methylisoscutellarein 7-O-[6'''-acetylallopyranosyl(1→2) glucopyranoside (**155**) and hypolaetin 8-glucoside (**140**) were isolated and identified. Two flavone glycosides both having C-8 oxydation have been obtained from *S. leucantha* [68], previously the aglycone 5,3',4'-trihydroxy-6,7,8-trimethoxyflavone (**154**) was isolated from the same plant [69], the two glycosides were 5,7,3',4'-tetrahydroxy-6,8-dimethoxyflavone 7-glucoside (**156**) and 5,7,4'-trihydroxy-6,8,3'-trimethoxyflavone 7-glucoside (**157**). In a survey study [70] using more than 30 Spanish *Sideritis* species, aglycones obtained from the ether extracts were apigenin (**18**), chrysoeriol (**17**), cirsiliol (**116**), sideritoflavone (**154**), cirsimaritin (**2**), cirsilineol (**9**), xanthomicrol (**132**). Among them sideritoflavone (**154**) was found to be the major component in eight *Sideritis* species.

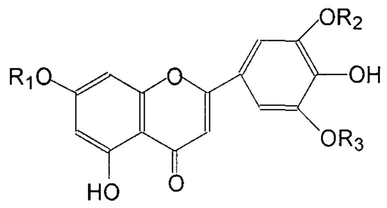
## *MENTHA* FLAVONOIDS

*Mentha longifolia* has yielded luteolin (**6**), hesperetin 7-rutinoside (**158**) and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (**159**) [71]. *M. suaveolens* and *M. pulegium* have yielded 5,7-dihydroxy-6-methoxyflavone (**57**) and 5,6-dihydroxy-7-methoxyflavone (**160**) [72]. *M. spicata* four methylated aglycones were obtained with antihistaminic activity, the compounds were 5-O-desmethylnobiletin (**147**), 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone (**161**), 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone (thymonin) (**162**) and 5,3',4'-trihydroxy-6,7,8-trimethoxyflavone (sideritoflavone) (**154**) [73]. In another study with *M. longifolia* [74], the plant has yielded both C- and O-glycosides, such as isoorientin (**163**), vicenin-2 (**44**), hypolaetin (**118**), lucenin-1 (**164**), luteolin 7-O-glucoside (**7**) and luteolin 7-O-neohesperidoside (= 2-O-α-L-rhamnosyl-D-glucose) (**165**). In this study three new flavone glycosides were obtained, tricetin 7-O-methyl ether 3'-O-glucoside-5'-O-rhamnoside (**166**), tricetin 3'-O-glucoside-5'-O-rhamnoside (**167**) and tricetin 3'-O-rhamnosyl(1→4)rhamnoside (**168**). From *M. longifolia* thymonin (**162**), luteolin (**6**), luteolin 7-O-glucoside (**7**), hesperidin (**158**) and a new flavone aglycone 5,7,4'-trihydroxy-6,2',3'-trimethoxyflavone (**169**) were obtained [75]. A new flavanone glycoside longitin (**170**) was isolated from *M. longifolia* [76], its structure was established using extensive spectral data. Three new flavonol glycosides

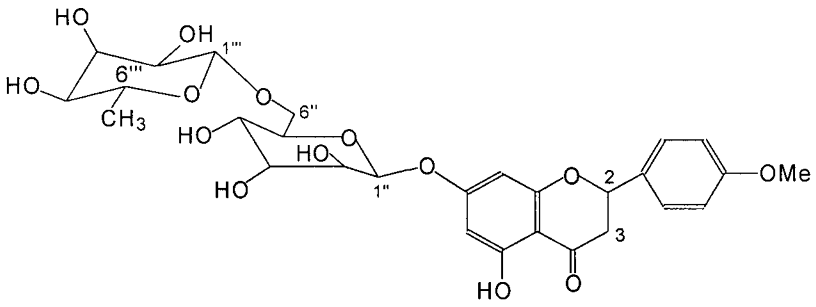
were isolated from *M. lavandulacea* [77], these were kaempferol 3-O-glucosyl(1→2) rhamnoside-7-O-glucoside (**171**), kaempferol 3-O-[6'''-p-coumarylglucosyl(1→2)rhamnoside-7-O-glucoside] (**172**) and kaempferol 3-O-[4'''-p-coumaroylglucosyl(1→2)rhamnoside-7-O-glucoside] (**173**).



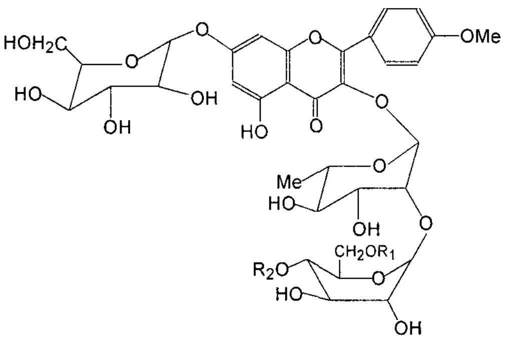

| | R | R6 | R7 | R8 | R2' | R3' | R4' |
|---|---|---|---|---|---|---|---|
| **159** | Me | OMe | OMe | H | H | H | OMe |
| **160** | H | OH | OMe | H | H | H | H |
| **161** | H | OH | OMe | OMe | H | Me | OMe |
| **162** | H | OH | OMe | OMe | H | Me | OH |
| **163** | H | C-Glc | OH | H | H | H | OH |
| **164** | H | C-Xyl | OH | C-Glc | H | H | OH |
| **165** | H | H | Rha-Glc | H | H | H | H |
| **169** | H | OMe | OH | H | OMe | Me | OH |




**166** $R_1$ = Me $R_2$ = Glucosyl $R_3$ = Rhamnosyl
**167** $R_1$ = H $R_2$ = Glucosyl $R_3$ = Rhamnosyl
**168** $R_1$ = H $R_2$ = Dirhamnosyl $R_3$ = H




**170**

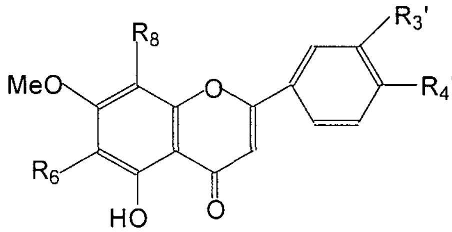



| | | |
|---|---|---|
| **171** | $R_1$= H | $R_2$= H |
| **172** | $R_1$= p-coumaroyl | $R_2$= H |
| **173** | $R_1$= H | $R_2$= p-coumaroyl |

## *THYMUS* FLAVONOIDS

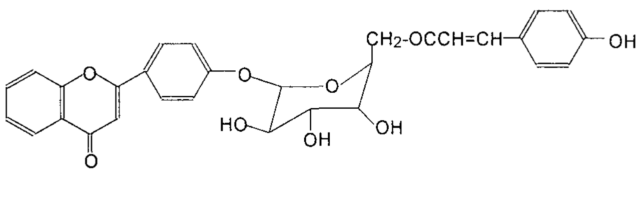
*Thymus piperella* has yielded a group of known and a new flavonoid 5,6-dihydroxy-7,8,4'-trimethoxyflavone (**174**), others were known compounds 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone (**161**), apigenin (**18**), flavanones naringenin (**175**), eriodictyol (**112**), vicenin-2 (**44**), apigenin 7-O-β-D-glucuronide (**20**) and luteolin 7-glucoside (**7**) [78]. Aerial parts of *T. herba barona* has yielded luteolin (**6**), sorbifolin (**48**), thymusin (**176**), cirsiliol (**116**), apigenin (**18**), sideritoflavone (**154**), cirsimaritin (**2**),

cirsilineol (**9**), xanthomicrol (**132**), 8-methoxycirsilineol (**153**) and genkwanin (**16**) as well as flavanones eriodictyol (**112**) and naringenin (**175**) [79]. Antioxidative flavones obtained from *T. vulgaris* were xanthomicrol (**132**), cirsilineol (**9**), genkwanin (**16**), 5-hydroxy-7,4'-dimethoxyflavone (**134**), 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone (**177**) and cirsimaritin (**2**) [80]. A new acylated flavonoid glycoside (**178**) was isolated from *T. serpyllum*, its structure was elucidated on the basis of spectral data and chemical degradation [81].




| | $R_6$ | $R_8$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|
| **174** | OH | OMe | H | OMe |
| **176** | OH | OMe | H | OH |
| **177** | OMe | OMe | OMe | OH |
| **179** | H | H | OH | OH |

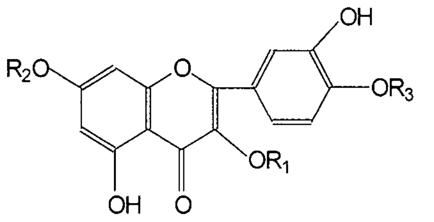
*T. satureioides* exhibited spasmolitic activity on smooth muscles. The compounds obtained were cirsilineol (**9**), 8-methoxycirsilineol (**153**), and thymonin (**162**) [82]. From *T. membranaceus* Boiss. *subsp. membranaceus* luteolin (**6**), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**4**), 5-desmethyl-nobiletin (**147**), eupatorin (**49**), sideritoflavone (**154**), chrysoeriol (**17**), 7-methoxyluteolin (**179**), genkwanin (**16**), xanthomicrol (**132**) and naringenin (**175**) [83].




**178**

## *PHLOMIS* FLAVONOIDS

One new and three known flavonol glycosides were obtained from the aerial parts of *Phlomis spectabilis* [84]. The new glycoside was 3-O-[6''-(E)-p-coumaroyl]-β-D-glucopyranoside of kaempferol 7,4'-dimethyl ether (**180**). The others were tiliroside (**181**), kaempferol-7,4'-dimethyl ether 3-glucoside (**182**) and astragalin (**127**).




| | | |
|---|---|---|
| **180** | $R_1$= 6''-O-p-coumarylglucoside | $R_2$ = $R_3$ = Me |
| **181** | $R_1$= 6''-O-p-coumaroylglucoside | $R_2$ = $R_3$ = H |
| **182** | $R_1$= Glucose | $R_2$ = $R_3$ = Me |

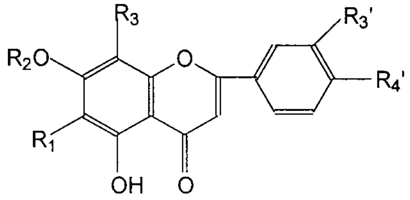
*P. aurea* Decne and *P. floccosa* D. Don were studied and similar flavones were obtained [85]. The compounds were apigenin 7-glucoside (**19**), apigenin 7-p-coumaroylglucoside (**144**), hispidulin 7-glucoside (**46**), luteolin 7-glucoside (**7**), luteolin 7-rutinoside (**183**), chrysoeriol 7-p-coumaroylglucoside (**184**), vicenin-2 (**44**), lucenin-2 (**185**), naringenin (**175**), naringenin 7-glucoside (**186**), naringenin 7-p-coumaroylglucoside (**187**). Quite similar flavone glycosides were isolated from *P. lychnitys,* such as luteolin (**6**), apigenin (**18**), chrysoeriol (**17**) and their 7-glucosides and p-coumaroylglucosides one of which being a new compound chrysoeriol 7-β-D-(3''-E-p-coumaroyl) glucoside (**184**) [86]. Two new flavone glycosides, luteolin 7-O-(6''-O-β-D-apiofuranosyl)-β-D-glucopyranoside (**188**) and chrysoeriol 7-O-(6''-O-β-D-apiofuranosyl)-β-glucopyranoside (**189**) were isolated from *P. nissolii* [87]. Two new compounds named as phlomis flavosides A (**190**) and B (**191**) were obtained together with astragalin (**127**) and isoquercitrin (**24**) from *P. spinidens* [88].

## *MARRIBIUM* FLAVONOIDS

From the leaves of *Marrubium vulgare* in addition to known flavonoids luteolin (**6**), apigenin (**18**), chrysoeriol (**17**), luteolin 7-glucoside (**7**), apigenin 7-glucoside (**19**), apigenin 7-(6''-p-coumaroyl) glucoside (**192**), new glycosides such as apigenin 7-lactate-2''-O-β-glucoside (**193**), luteolin 7-lactate (**194**), luteolin 7-lactate-2''-O-β-glucuronide (**195**) were isolated, their structures were established by extensive NMR techniques [89]. *M. alysson* which is a medicinal plant in Egypt has yielded apigenin (**18**) and its 7-glucoside (**19**) and arabinoside (**196**) [90].

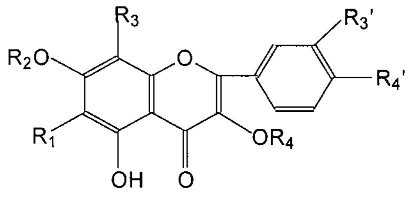
## *LYCOPUS* FLAVONOIDS

*Lycopus virginicus* has yielded six flavonoids apigenin 7,4'-dimethyl ether (**134**), luteolin 7,4'-dimethyl ether (**15**), genkwanin (**16**), apigenin (**18**), acacetin 7-O-β-D-(6''-O-methyl)glucuronide (**197**) and apigenin 7-O-β-D-glucoside (**19**) [91]. A further study with the same plant by the same group has yielded more glycosides of apigenin, apigenin 7-O-glucuronide (**20**), acacetin 7-O-glucuronide (**198**), luteolin 7-O-glucuronide (**21**) and apigenin 7-O-β-D-(6''-O-methyl)glucuronide (**199**) [92]. Flavone aglycones apigenin (**18**), luteolin (**6**), genkwanin (**16**), 5,3',4'-trihydroxy-7-methoxyflavone (**23**), and cirsiliol (**116**) were isolated from the aerial parts of *L. europaeus* L. [93]. From the same plant flavone and flavonol glycosides were also obtained, these are eriodictyol 7-O-β-D-glucoside (**200**), eriodictyol 7-O-rutinoside (**201**), acacetin 7-O-neohesperidoside (**202**), isoscutellarein 7-O-(6'''-O-acetyl-2''-O-β-D-allosyl)-β-D-glucoside (**119**), quercitrin (**203**), isoquercitrin (**24**), hyperin (**204**), rutin (**122**) [94]. The presence of antigonadotropic activity has been proven in *L. europaeus* L. among other plants, although phenolic compounds show this activity, while luteolin 7-glucuronide (**21**) induced a slight activity [95].




| | $R_1$ | $R_2$ | $R_3$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|
| **183** | H | Rut | H | OH | OH |
| **184** | H | Cou-Glc | H | OMe | OH |
| **185** | C-Glc | H | C-Glc | OH | OH |
| **188** | H | Api-Glc | H | OH | OH |
| **189** | H | Api-Glc | H | OMe | OH |
| **190** | H | H | H | OH | O-Api |
| **191** | H | H | H | H | O-Api |
| **192** | H | Cou-Glc | H | H | OH |
| **193** | H | Lac-Glc | H | H | OH |
| **195** | H | Lac-Glucu | H | OH | OH |
| **196** | H | Ara | H | H | OH |
| **197** | H | Me-Glucu | H | H | OMe |
| **198** | H | Glucu | H | H | OMe |
| **199** | H | Me-Glucu | H | H | OH |
| **202** | H | Rha-Glc | H | H | OMe |
| **211** | OH | Me | H | OMe | OH |

Api-Glc = Apiofuranosyl-glucopyranoside
Lac-Glc = Lactate-glucose
Lac-Glucu = Lactate-glucuronide
Rha-Glc = Rhamnoglucoside



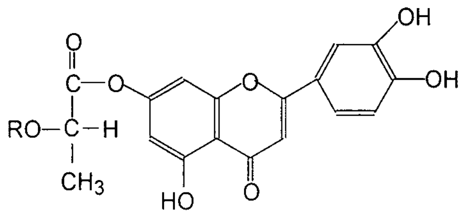

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|---|
| **203** | H | H | H | Rha | OH | OH |
| **204** | H | H | H | Galac | OH | OH |

## *NEPETA* FLAVONOIDS

From *Nepeta hindostana* [96] a new flavone glycoside nepitrin (**47**) was isolated. *Nepeta transcaucasica* has yielded external and vacuolar flavonoids [97], these were cirsimaritin (**2**), xanthomicrol (**132**), salvigenin (**1**), gardenin B (**131**), apigenin (**18**) and genkwanin (**16**).

## *BALLOTA* FLAVONOIDS

Two interesting flavonoids, luteolin 7-lactate (**194**) and luteolin 7-glucosyl-lactate (**205**) were isolated from *Ballota nigra* a commonly distributed plant in western Europe [98].



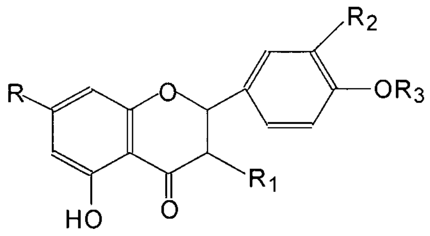

**194** **R** = H
**205** **R** = β-D-glucose

Previously, from the same plant apigenin 7-glucoside (**19**) and vicenin-2 (**44**) were isolated [99]. From *B. saxatilis* subsp. *saxatilis* ladanein (**130**), kaempferol-3,7,4'-trimethyl ether (**150**), quercetin-3,7,3',4'-tetramethyl ether (**206**) have been isolated [100].

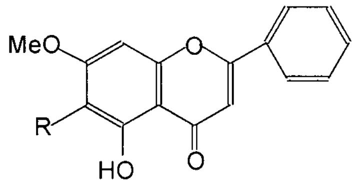
## *OREGANO* FLAVONOIDS

*Origanum vulgare* L. leaves consisted apigenin (**18**), a flavanone eriodictyol (**112**) and dihydroflavonols, dihydrokaempferol (**207**) and dihydroquercetin (**208**) [101]. A new flavone glycoside was isolated from *O. vulgare* L. together with some known flavones. The new compound was 5-hydroxy-7-methoxy-6-O-[α-L-rhamnopyranosyl(1→2)-β-D-fuco-

pyranosyl] flavone (**209**) [102]. *O. compactum* has yielded a rare flavanonol (2R,3R)-3,5,7,4'-tetrahydroxyflavanone, aromadendrin (**210**) [103]. From the glandular hairs of *O. x intercedens* flavone aglycones thymusin (**176**), thymonin (**162**), cirsimaritin (**2**), genkwanin (**16**) and 5,6,4'-trihydroxy-7,3'-dimethoxyflavone (**211**) were isolated [104]. While the Mecadonian collection of the same plant has yielded luteolin (**6**) [105].



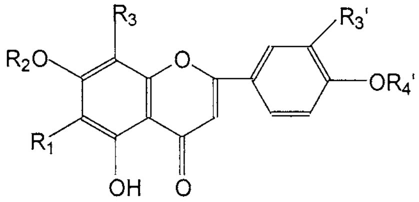

| | R | R1 | R2 | R3 |
|---|---|---|---|---|
| **200** | O-Glc | H | H | H |
| **201** | O-Rut | H | H | H |
| **206** | OMe | OMe | OMe | Me |
| **207** | H | OH | H | H |
| **208** | H | OH | OH | H |
| **210** | OH | OH | H | H |



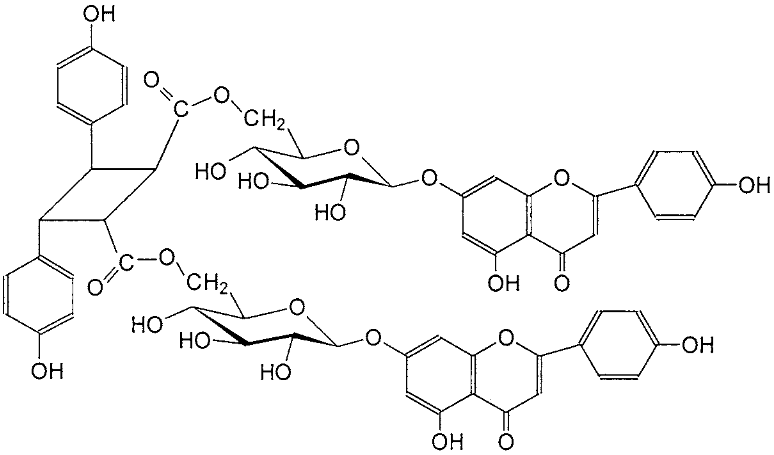

**209** **R** = α-L-Rhamnopyranosyl-(1→2)-β-D-fucopyranosyl

# *STACHYS* FLAVONOIDS

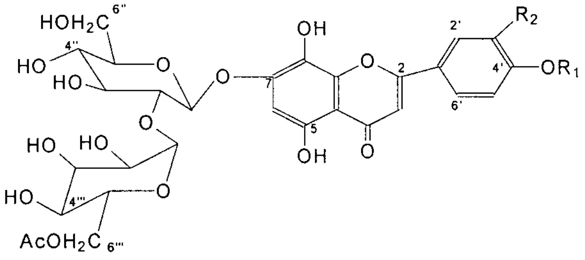
*Stachys chrysantha* and *S. candida* have yielded xanthomicrol (**132**) and chrysoeriol-7-O-β-D-(3''-E-p-coumaroyl)-glucopyranoside (**184**) which were active compounds [106]. From *Stachys spinosa* together with three iridoids a new flavone glycoside stachyspinoside (**212**) was isolated, its structure was decided by extensive NMR techniques [107]. *S. aegyptiaca* [108] has yielded vicenin-2 (**44**), lucenin-2 (**185**), apigenin (**18**), xanthomicrol (**132**), luteolin 7-glucoside (**7**) and a new flavone glycoside isoscutellarein 8-O-(6''-trans-p-coumaroyl)-β-D-glucoside (**213**). In another study with the same plant [109] twenty four flavonoids were isolated, twelve of them were identified as glycosides vicenin-2 (**44**), lucenin-2 (**185**), apigenin 7-glucoside (**19**), luteonin 7-glucoside (**7**), chrysoeriol 7-glucoside (**23**), isoscutellarein 7-allosyl(1→2)glucoside (**214**), isoscutellarein 7-[6'''-acetylallosyl(1→2)]glucoside (**119**), hypolaetin 7-[6'''-acetylallosyl (1→2)-3''-acetylglucoside] (**215**), apigenin 7-[6'''-acetylallosyl(1→2)glucoside] (**216**) and luteolin 7-[6'''-acetylallosyl (1→2) glucoside] (**217**) and flavone aglycones apigenin (**18**), luteolin (**6**), chrysoeriol (**17**), xanthomicrol (**132**), 5,4'-dihydroxy-6,7,8,3'-tetrameth-oxyflavone (**218**) and other methoxylated flavones. The same plant has yielded a unique acylated flavone diapigenin 7-O-(6''-trans,6''-cis-p,p'-dihydroxy-μ-truxinyl) glucoside stachysetin (**219**) together with known compounds. The structure of **219** was decided by extensive NMR data [110]. From *S. recta* [111] three flavone glycosides were obtained as 4'-O-methylisoscutellarein 7-O-(2''-O-6'''-O-acetyl-β-D-allopyranosyl-β-D-glucopyranoside) (**220**), isoscutellarein 7-O-(2''-O-6'''-O-acetyl-β-D-allopyranosyl-β-D-glucopyranoside) (**119**), 3'-hydroxy-4'-methyliso-scutellarein 7-O-(2''-O-6'''-O-acetyl-β-D-allopyranosyl-β-D-glucopyra-noside) (**155**). Compounds **219** and **155** together with four new allose containing flavonoid glycosides were obtained from *S. anisochila* [112] these were hypolaetin 7-O-[6'''-O-acetyl-β-D-allopyranosyl-(1→2)-β-D-glucopyranoside] (**221**), isoscutellarein 7-O-[6'''-O-acetyl-β-D-allopyra-nosyl-(1→2)–6''-O-acetyl-β-D-glucopyranoside (**222**), 3'-hydroxy-4'-O-methylisoscutellarein 7-O-[6'''-O-acetyl-β-D-allopyranosyl-(1→2)-6''-O-acetyl-β-D-glucopyranoside] (**155**) and hypolaetin 7-O-[6'''-O-acetyl-β-D-allopyranosyl-(1→2)-6''-O-acetyl-β-D-glucopyranoside] (**223**).



| | $R_1$ | $R_2$ | $R_3$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|
| **213** | H | H | OCouGlc | H | H |
| **214** | H | AlloGlc | OH | H | H |
| **215** | H | AcAlloAcGlc | H | OH | H |
| **216** | H | AcAlloGlc | H | H | H |
| **217** | H | AlloGlc | H | OH | H |
| **218** | OMe | Me | OMe | OMe | OH |



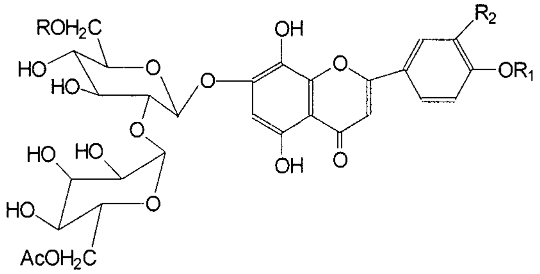
**219**




| | $R_1$ | $R_2$ |
|---|---|---|
| **119** | H | H |
| **155** | Me | OH |
| **220** | Me | H |

## FLAVONOIDS FROM MISCELLANEOUS GENERA

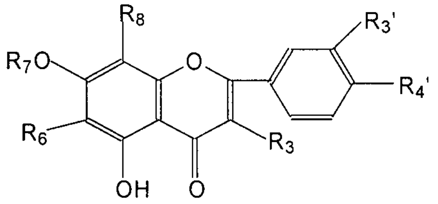
Genus *Thymbra* is not a well investigated for flavonoidal compounds, however *T. capitata* has yielded a number of 6-hydroxy flavonoids, 5,6,4'-trihydroxy-7,3'-dimethoxyflavone (**211**), 5,6-dihydroxy-7,3',4'-trimethoxyflavone (**224**), luteolin (**6**), diosmetin (**10**), vicenin-2 (**44**) and luteolin 7-rutinoside (**183**) [113]. The presence of 6-hydroxyflavones in *Thymbra* species was first shown in *T. spicata* which has yielded 6-hydroxyluteolin 7,3'dimethyl ether (**225**) together with luteolin (**6**) and rhamnetin (**226**) [114]. There is only one flavone study in *Ajuga* genus *A. chia* which yielded apigenin (**18**), luteolin (**6**) and luteolin 7-glucosides (**7**) [115]. In *Lavandula* species 8-hydroxyflavones were found to be present. Hypolaetin 8-glucuronide (**136**), hypolaetin 4-methyl ether 8-O-glucuronide (**137**), isoscutellarein 8-O-glucuronide (**227**) were isolated from *Lavandula coronopifolia* and *L. pubescens* [116]. Only one species of genus *Hyssopus* contain flavonoids, in *H. officinalis* [117] the major flavone was found to be diosmin (**228**). The flowers of *Lamium album* has also yielded flavonoid p-coumaroylglucosides [118]. *Betonica officinalis, B. bulgarica* Deg. et Neic. and *B. sardinica* Griseb. were screened and the presence of flavonoid glycosides were established [119].



| | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **221** | H | H | H |
| **222** | Ac | H | H |
| **223** | H | Me | OH |

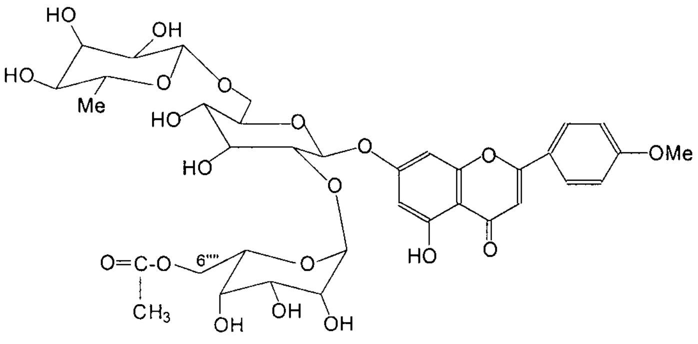
An acetylated flavone glycoside acacetin 7-O-[6''''-O-acetylglucosyl (1''''→2'')]-rhamnosyl (1'''→6'')glucoside (**229**) has been isolated from the leaves of *Calamintha glandulosa* together with acacetin 7-O-rhamnosyl (1''''→6'') glucoside linarin (**230**), the same compounds were obtained from *Satujera, Micromeria, Acinos* and *Clinopodium* genera indicating a possible taxonomic significan [120]. *Calamintha incana* has yielded a new flavone glycoside alquds (**231**) which is identified by 1D and 2D NMR and UV, IR spectroscopy [121]. In a taxonomic study of European *Galeopsis* species the presence of some flavonoids was established. In *G. angustifolia* hypolaetin (**118**), isoscutellarein (**232**) and hypolaetin 4'-methyl ether 7-O-β-D-(2''-O-β-D-allosyl)glucosides (**233**) were found, and from *G. accumutates* luteolin (**6**), apigenin (**18**), scutellarein 7-O-β-D-glucoside (**75**) and coumaroyl glucosides of apigenin and luteolin were isolated [122]. There is only one study in the genus *Prunella, P. vulgaris* has yielded luteolin (**6**) and cinaroside (**7**) [123]. A new glycoside isosakuranetin 7-O-rhamnoside (**234**) was isolated from *Cyclotrichium niveum* together with apigenin (**18**), genkwanin (**16**), acacetin 7-O-rutinoside (**235**), this is the only flavonoidal investigation of this genus [124]. There is also only one study with one of *Dracocephalum* species, *D. nutans* which afforded apigenin 7-glucoside (**19**) and luteolin 7-glucoside (**7**) [125]. There are some flavonoidal investigations of *Leonurus* species. From *L. cardiaca* isoquercitrin (**24**), hyperoside (**204**), quercitrin (**203**), quercetin 7-O-glucoside (**236**), kaempferol 3-O-glucoside (**127**), apigenin 7-O-D-glucoside (**19**) and apigenin 5-O-D-glucoside (**125**)

as well as apigenin 4'-O-D-glucoside (**237**) were isolated [126]. Tomas Barberan et al. in a comprehensive study of *Leonurus* species detected flavonoid p-coumaroyl glycosides such as apigenin 7-p-coumaroyl glucoside (**144**), and genkwanin (**16**) [127].

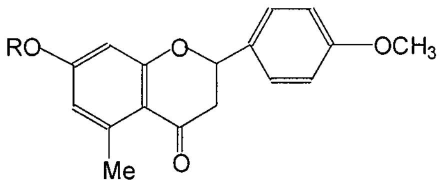



| | $R_3$ | $R_6$ | $R_7$ | $R_8$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|---|
| **224** | H | OH | Me | H | OMe | OH |
| **225** | H | OH | Me | H | OMe | OH |
| **226** | OH | H | Me | H | OH | OH |
| **227** | H | H | H | O-Glucu | H | OH |
| **228** | H | H | Rut | H | OH | OMe |
| **229** | H | H | AcGlc-RhaGlc | H | H | OMe |
| **230** | H | H | Rha-Glc | H | H | OMe |
| **232** | H | H | H | OH | H | OH |
| **233** | H | H | Allo-Glc | OH | OH | OMe |
| **235** | H | H | Rut | H | H | OMe |
| **236** | OH | H | Glc | H | OH | OH |
| **237** | H | H | H | OH | H | O-Glc |
| **238** | OH | H | Me | H | H | OH |
| **239** | OH | H | H | H | H | OH |
| **240** | OH | H | H | H | OH | OH |

From *Melissa officinalis* subsp. *officinalis* luteolin 3'-O-β-D-glucuronide (**43**) was isolated [128]. In another study with the same plant luteolin 7-O-glucoside (**7**), isoquercitrin (**24**), apigenin 7-O-glucoside (**19**) and rhamnocitrin (**238**) were isolated [129].

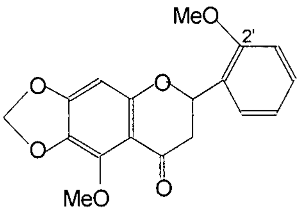



**229**

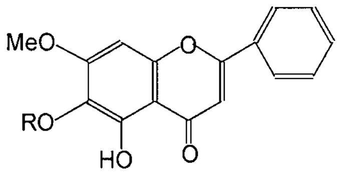



| | R |
|---|---|
| **234** | Rha |
| **241** | Rut |

Vasorelaxant effect of three flavonoids from *Satujera obovata* was studied, these were luteolin (**6**), eriodictyol (**112**) and naringenin (**182**) [130], as well as vasodilatory activity [131].



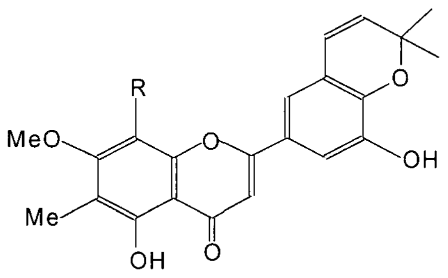

**243**



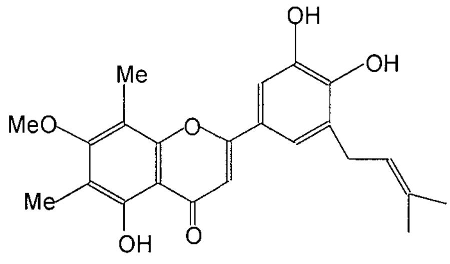

**244**

R=α-L-Rham(1→2)β-D-fucopyrglc.

From *Acinos suaveolens* apigenin (**18**), kaempferol (**239**), quercetin (**240**), chrysoeriol (**17**), luteolin (**6**), didymin (**241**), narirutin (**242**), apigenin 7-glucoside (cosmoiin) (**19**), cynaroside (**7**), isoquercitrin (**24**), hesperidine (**158**) and luteolin 7-O-rutinoside (**183**) were isolated [132]. From *Elsholtzia blanda* (Benth.) Benth 5,2'-dimethoxy-6,7-methylenedioxy-flavanone (**243**), 5-hydroxy-7-methoxy-6-O-[α-L-rhamnopyranosyl (1→2) β-D-fucopyranosyl] flavone glycoside (**244**) were isolated together with some other compounds [133]. Three new C-methylated flavones were isolated from *Elsholtzia stauntonii* in China [134], these were muxiangrine I (**245**), II (**246**) and III (**247**).




**245** **R** = Me
**246** **R** = H

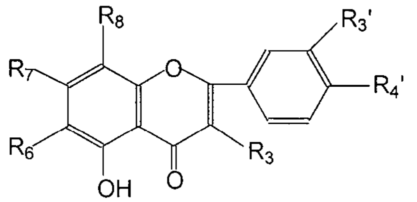



**247**

# SELECTED SPECTRA OF LABIATAE FLAVONOIDS

*Ultraviolet Spectra*

Using the shift reagents ultraviolet spectral data give important information about the flavonoid structures. The following data give structural information of simple and substituted flavonoids. Table 6 gives the UV data of apigenin (**18**), luteolin (**6**) and their methyl derivatives such as genkwanin (**16**), salvigenin (**1**), cirsimaritin (**2**), xanthomicrol (**132**), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**4**), hypolaetin (**118**), thymonine (**162**) and thymusin (**176**), 6-hydroxyluteolin-7,3',4'-trimethyl ether (**224**), 6-hydroxyluteolin-7,3'-dimethyl ether (**225**). Table 7 shows flavonols and their methylated derivatives quercetin-3,3'-dimethyl ether (**29**), kaempferol (**239**), quercetin (**240**), kaempferol-3,6,4'-methyl ether (**248**), quercetin 3,7-diglucoside (**249**),. The UV spectra of flavone and flavonol glycosides apigenin 7-glucoside (**19**), luteolin 7-glucoside (**7**), hypolaetin 8-glucoside (**140**), kaempferol 7-glucoside (**250**), kaempferol 3-glucoside (**127**), quercetin 3-glucoside (**24**), quercetin 3,7-diglucoside (**249**), jacein (**251**), gossypin (**252**) are given in Table 8. UV spectra of flavanone and their glycosides eriodictyol (**112**), hesperidin (**158**), naringenin (**175**), isosakuranetin 7-O-rhamnoglucoside (**234**) are given in Table 9.




| | $R_3$ | $R_6$ | $R_7$ | $R_8$ | $R_{3'}$ | $R_{4'}$ |
|---|---|---|---|---|---|---|
| **248** | OMe | OMe | OH | H | OH | OH |
| **249** | O-Glc | H | O-Glc | H | OH | OH |
| **250** | OH | H | O-Glc | H | H | OH |
| **251** | OMe | OMe | O-Glc | H | OMe | OH |
| **252** | OH | H | OH | O-Glc | OH | OH |

Table 4. A selection of flavone and flavonol $^{13}$C-NMR spectra (solvent; DMSO-$d_4$)

| Flavones | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ | $C_7$ | $C_8$ | $C_9$ | $C_{10}$ | $C_{1'}$ | $C_{2'}$ | $C_{3'}$ | $C_{4'}$ | $C_{5'}$ | $C_{6'}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flavone ** | 162.1 | 106.7 | 176.7 | 125.1 | 124.5 | 133.8 | 118.2 | 155.3 | 123.1 | 130.8 | 126.0 | 128.7 | 131.4 | 128.7 | 126.0 |
| 5-OH flavone * | 164.1 | 105.6 | 182.9 | 159.8 | 110.8 | 135.6 | 107.2 | 155.9 | 110.1 | 130.5 | 126.4 | 128.9 | 132.0 | 128.9 | 126.4 |
| 5,4'-OH flavone * | 165.5 | 103.9 | 183.5 | 160.0 | 111.4 | 136.2 | 108.0 | 156.4 | 110.5 | 121.9 | 129.2 | 116.7 | 161.7 | 116.7 | 129.2 |
| 5,7-OH flavone | 163.0 | 105.0 | 181.6 | 161.5 | 98.9 | 164.3 | 94.0 | 157.3 | 103.9 | 131.7 | 126.0 | 128.0 | 130.6 | 128.0 | 126.0 |
| Apigenin | 164.1 | 102.8 | 181.8 | 161.5 | 98.8 | 163.7 | 94.0 | 157.3 | 103.7 | 121.3 | 128.4 | 116.0 | 161.1 | 116.0 | 128.4 |
| Acacetin | 163.9 | 103.9 | 182.3 | 162.2 | 99.4 | 164.8 | 94.3 | 157.9 | 104.4 | 123.5 | 128.4 | 114.8 | 162.8 | 114.8 | 128.4 |
| 5-Me Apigenin | 160.4† | 107.1 | 175.7 | 159.9† | 96.4 | 162.4 | 95.2 | 159.0† | 106.0 | 121.5 | 127.7 | 115.8 | 160.6 | 115.8 | 127.7 |
| 7-Me Apigenin | 164.6 | 103.4 | 182.3 | 161.8 | 98.2 | 165.6 | 92.9 | 157.7 | 105.0 | 121.6 | 128.8 | 116.3 | 161.8 | 116.3 | 128.8 |
| Luteolin | 164.5† | 103.3 | 182.2 | 162.1 | 99.2 | 164.7† | 94.2 | 157.9 | 104.2 | 122.1 | 113.8 | 146.2 | 150.1 | 116.4 | 119.3†† |
| Chrysoeriol | 163.7† | 103.8 | 181.8 | 161.6 | 98.8 | 164.2† | 94.0 | 157.4 | 103.3 | 121.7 | 110.2 | 150.8 | 148.0 | 115.8 | 120.4†† |
| Diosmetin | 163.6 | 104.0 | 181.8 | 161.7 | 99.0 | 164.4 | 94.0 | 157.5 | 103.7 | 123.3 | 113.1 | 146.9 | 151.2 | 112.1 | 118.7†† |
| Tricretin * | 164.2 | 103.2 | 181.6 | 161.6 | 99.0 | 164.2 | 93.9 | 157.5 | 104.0 | 120.9 | 106.0 | 146.5 | 137.9 | 146.5 | 106.0 |
| Flavonols | | | | | | | | | | | | | | | |
| Kaempferol | 146.8 | 135.6 | 175.9 | 160.7 | 98.2 | 163.9 | 93.5 | 156.2 | 103.1 | 121.7 | 129.5 | 115.4 | 159.2 | 115.4 | 129.5 |
| Quercetin | 146.9 | 135.8 | 175.9 | 160.8 | 98.3 | 164.0 | 93.5 | 156.2 | 103.1 | 122.1 | 115.2 | 145.1 | 147.7 | 115.7 | 120.1 |
| 5-Me quercetin | 142.0 | 137.1 | 171.1 | 160.6 | 96.0 | 162.6 | 94.8 | 158.1 | 105.2 | 122.4 | 114.6 | 145.1 | 147.1 | 115.7 | 119.3 |
| 7-Me quercetin | 147.3 | 136.0 | 175.9 | 160.4 | 97.4 | 164.9 | 91.9 | 156.0 | 103.7 | 121.9 | 115.2 | 145.0 | 147.8 | 115.6 | 120.1 |
| 4',7-Me quercetin | 146.7 | 136.4 | 176.0 | 160.4 | 97.4 | 164.9 | 91.8 | 156.0 | 104.0 | 123.4 | 114.8 | 146.7 | 149.4 | 111.7 | 119.8 |
| Morin * | 149.6 | 136.5 | 176.7 | 161.3 | 99.6 | 164.1 | 95.0 | 156.9 | 105.0 | 111.2 | 158.1 | 105.0 | 161.0 | 109.3 | 132.3 |
| Myricetin (3-*O*-glyc) * | - | - | - | 161.2 | 98.6 | 164.0 | 93.3 | 156.2 | 104.0 | 120.0 | 108.8 | 145.3 | 136.6 | 145.2 | 108.8 |

† Assignments may be reversed.
†† Assignments for C-1' and C-6' reversed according to Linuma *et al.* (1980).
* Spectra from Ternai and Markham (1976) or Markham *et al.* (1978). All others from Wagner *et al.* (1976).
** Spectra from Wenkert and Gottlieb (1977).

Table 5. $^{13}C$-NMR spectra of selected monosaccharides in $D_2O$ solvent

| Sugar | | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
|---|---|---|---|---|---|---|---|
| **D-Glucopyranose** | α | 92.7 | 72.1 | 73.4 | 70.4 | 72.1 | 61.3 |
| | β | 96.5 | 74.8 | 76.4 | 70.3 | 76.6 | 61.5 |
| **D-Galactopyranose** | α | 93.6 | 69.8 | 70.6 | 70.6 | 71.7 | 62.5 |
| | β | 97.7 | 73.7 | 74.2 | 70.1 | 76.3 | 62.3 |
| **D-Mannopyranose** | α | 95.0 | 71.7 | 71.3 | 68.0 | 73.4 | 62.1 |
| | β | 94.6 | 72.3 | 74.1 | 67.8 | 77.2 | 62.1 |
| **D-Xylopyranose** | α | 93.3 | 72.5 | 73.9 | 70.4 | 62.1 | |
| | β | 97.6 | 75.1 | 76.9 | 70.3 | 66.3 | |
| **L-(or D) Arabinopyranose** | α | 97.8 | 73.0 | 73.5 | 69.6 | 67.5 | |
| | β | 93.7 | 69.6 | 69.8 | 69.8 | 63.6 | |
| **Rhamnopyranose** | α | 95.0 | 71.9 | 71.1 | 73.3 | 69.4 | 18.0 |
| | | 94.6 | 72.4 | 73.8 | 72.9 | 73.1 | 18.0 |

Chemical shifts expressed in ppm from TMS

**Table 6. UV Spectral data of some flavones ($\lambda_{max}$, nm)**

| Compd. | MeOH | NaOAc | NaOAc-$H_3BO_3$ | NaOMe | $AlCl_3$ | $AlCl_3$ – HCl |
|---|---|---|---|---|---|---|
| **1** | 330,278 | 370,276 | 333,275 | 372*,325*,298 | 353,295 | 348,298 |
| **2** | 276, 333 | 274, 341, 381 | 275, 334 | 273, 290*,383 | 261*, 285*, 301,362 | 258, 299, 360 |
| **6** | 240*, 253, 267, 349 | 268, 325*, 384 | 262, 305*, 370, 426* | 266*, 330*, 400 | 272, 300*, 330, 422 | 268*, 275, 295*,355, 385 |
| **16** | 267, 300*, 333 | 267, 290*, 380 | 267, 309*, 337 | 273, 300, 385 | 275, 301, 349, 382 | 272, 300, 382 |
| **18** | 266, 296*, 335 | 272, 300, 373 | 270, 300*, 340 | 275, 321, 390 | 277, 304, 348, 384 | 276, 300 |
| **118** | 260, 330*, 380 | 80, 330, 400 (dec.) | 267, 280*, 325, 400 | 245*, 300*,330, 430 (dec.) | 260*, 275, 365*,452 | 270, 310*, 367, 441 |
| **132** | 282, 292*, 334 | 275, 340*, 390 | 275, 295*, 340 | 279, 365*, 340 | 267*, 290, 363,405* | 265*, 290, 354,405* |
| **162** | 236, 296, 341 | 290*, 308,356, 404 | 298*, 310, 338 | 239, 396 | 241, 261*, 307, 378 | 240, 261*,310, 376 |
| **176** | 242, 297, 332 | 302, 335, 379 | 301, 335, 385* | 284*, 339, 337 (dec.) | 313, 368 | 242, 297, 380* |
| **224** | 270*, 282, 340 | 270*, 289, 329 | 272*, 282 | 272*, 288, 316 | 275, 290, 370 | 262, 295, 370 |
| **225** | 72*, 282, 343 | 279, 330, 398 | 270*, 282,342 | 275, 300*, 394 | 275, 293, 373 | 275, 290, 370 |

* shoulder

Table 7. UV spectral data of some flavonols ($\lambda_{max}$, nm)

| Compd. | MeOH | NaOAc | NaOAc-$H_3BO_3$ | NaOMe | $AlCl_3$ | $AlCl_3$ – HCl |
|---|---|---|---|---|---|---|
| **29** | 257, 270*,360 | 272, 325, 385 | 260, 300*, 378 | 272, 330, 407 | 280, 302, 336, 442 | 270, 290*, 360, 400 |
| **239** | 250*,266,290,335 | 275, 300, 390 | 267, 297*, 372 | 278, 320, 415 (dec) | 260*, 265, 350, 424 | 260*, 268, 350, 422 |
| **240** | 255, 270*,370 | 255, 275, 370, 390 (dec) | 260, 300*, 390 | 250*, 320 (dec) | 270, 300*, 330, 458 | 268, 300*, 359, 430 |
| **248** | 256,270*,357 | 275, 315, 387 | 257, 270*, 360 | 270, 327, 415 | 267, 300*, 360, 403 | 275, 316, 390 |
| **249** | 250*, 267,367 | 260*, 275, 384 | 268, 300*, 320, 367 | 280, 320*, 411 | 250*, 270, 350, 423 | 250*, 270, 347, 420 |

* shoulder

Table 8. UV Spectral data of some flavone and flavonol glycosides ($\lambda_{max}$, nm)

| Compd. | MeOH | NaOAc | NaOAc-$H_3BO_3$ | NaOMe | $AlCl_3$ | $AlCl_3$ – HCl |
|---|---|---|---|---|---|---|
| 7 | 255, 267, 346 | 259, 265, 360, 398 | 260, 370 | 264, 300, 398 | 272, 300*, 330, 430 | 272, 300*, 330, 400 |
| **19** | 269, 333 | 267, 355, 385 | 268, 337 | 270, 389 | 275, 302, 345, 385 | 275, 302, 343, 383 |
| **24** | 260, 299*, 362 | 274, 324, 380 | 260, 300*, 377 | 270, 330, 409 | 275, 330*, 438 | 270, 300*, 365*, 405 |
| **127** | 250, 265, 350 | 265, 320*, 360, 405* | 265*, 320*, 360 | 245, 270, 350*, 390 | 255*, 275, 354, 400 | 274, 300*, 350, 398 |
| **140** | 258, 270*, 341 | 278, 323, 394 | 265, 277*, 382 | 272, 333*, 411 | 272, 302*, 332*, 432 | 270, 300*, 330*, 400 |
| **249** | 255, 270*, 355 | 261, 300*, 370, 400* | 260, 380 | 270, 300*, 400 | 275, 300*, 335, 440 | 270, 300*, 360*, 402 |
| **250** | 253, 265, 323, 364 | 260, 323, 385, 420* | 265*, 325*, 370 | 245, 267, 330*, 425 (dec.) | 260*, 266, 353, 424 | 258*, 270, 300*, 350, 422 |
| **251** | 257, 270*, 352 | 260, 370*, 420 | 257, 270*, 356 | 248, 270, 400 | 270, 300*, 387 | 267, 300*, 370, 405* |
| **252** | 260, 273*, 328*, 380 | 280, 328, 400 (dec.) | 267, 280*, 325, 400 | 245*, 300*, 330, 430 (dec.) | 260*, 275, 360*, 452 | 269, 310*, 367, 440 |

*shoulder

**Table 9. UV spectral data of some flavanones ($\lambda_{max}$, nm)**

| Compd. | MeOH | NaOAc | NaOAc-$H_3BO_3$ | NaOMe | $AlCl_3$ | $AlCl_3$ – HCl |
|---|---|---|---|---|---|---|
| **112** | 289,324* | 289,325 | 289,333 | 246,324 | 310,376 | 310,370 |
| **158** | 283, 326 | 284, 328 | 284, 326 | 242, 286, 356 | 308, 383 | 306, 380 |
| **175** | 287, 327* | 285*, 322 | 288, 322* | 325, 395* | 288, 322* | 282, 320* |
| **234** | 282, 324* | 283, 334* | 282, 324* | 282, 330* | 260, 312, 372 | 260, 300, 352 |

* shoulder

## *¹H NMR Spectra*

The information obtained from ¹H NMR spectra of flavonoids indicates the skeleton protons and attached groups such as methoxyl and sugar or some acyl groups. Table 10 gives the ¹H NMR data of some simple and methylated flavones apigenin (**18**), luteolin (**6**), cirsimaritin (**2**), genkwanin (**16**) and thymonin (**162**).

**Table 10. ¹H NMR Data of Some Flavones (δ)**

| | 18 | 6 | 2 | 16 | 162 |
|---|---|---|---|---|---|
| H-3 | 6.38s | 6.42s | 6.61s | 6.53s | 6.63s |
| H-6 | 6.15d | 6.18d | - | 6.27d | - |
| H-8 | 6.45d | 6.35d | 6.78s | 6.59d | - |
| H-2' | 7.80d | 7.30brs | 7.82d | 7.77d | 7.52d |
| H-3' | 6.80d | - | 6.84d | 6.81d | - |
| H-4' | - | - | - | - | - |
| H-5' | 6.80d | 6.85d | 6.84d | 6.81d | 6.91d |
| H-6' | 7.80d | 7.40dd | 7.82d | 7.77d | 7.52d |
| OMe | - | - | 3.92s | 3.83s | 4.02s |
| OMe | - | - | 3.81s | - | 3.94s |
| OMe | - | - | - | - | 3.92s |

¹H NMR data of flavone and flavonol glycosides are given in Table 11, luteolin 7-glucoside (**7**), apigenin 7-glucoside (**19**), hypolaetin 8-O-glucoside (**140**), 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone 2'-O-β-D-glucopyranoside (**253**), 5,2',6'-trihydroxy-6,7-dimethoxyflavone 2'-O-β-D-glucopyranoside (**254**), viscidulin III 2'-O-β-D-glucopyranoside (**111**).

**Table 11. ¹H NMR Data of Flavone and Flavonol Glucosides (δ)**

| | 7 | 19 | 140 | 253 | 254 | 111 |
|---|---|---|---|---|---|---|
| H-3 | 6.30s | 6.35s | 6.23s | 6.35s | 6.35s | 6.33s |
| H-6 | 6.25d | 6.30d | 6.66s | - | - | 6.31s |
| H-8 | 6.62d | 6.72d | - | - | 6.41s | - |
| H-2' | 7.30s | 7.70d | 7.61d | | - | - |
| H-3' | - | 6.80d | - | 6.79d | 6.74s | 6.92d |
| H-4' | - | - | - | 7.25t | 7.22t | 7.00d |
| H-5' | 6.82d | 6.80d | 6.89d | 6.69d | 6.67d | - |
| H-6' | 7.40dd | 7.70d | 7.75dd | - | - | - |
| G-1 | 4.95brd | 5.05d | 4.55d | 5.00d | 4.98d | 4.77d |
| G-2 | 3.4-3.8 | 3.3-4.0 | 3.36-3.73 | 3.20-3.50 | 3.19-3.50 | 3.05d |
| G-3 | " | " | " | " | " | 3.20t |
| G-4 | " | " | " | " | " | 3.25d |
| G-5 | " | " | " | 3.62dd | 3.58dd | 3.44d |
| G-6 | " | " | " | 3.75dd | 3.78dd | 3.68d |
| OMe | - | - | - | 3.81s | 3.85s | 3.72s |
| OMe | - | - | - | 3.82s | 3.85s | 3.76s |
| OMe | - | - | - | 4.00s | - | - |

$^{1}H$ NMR spectral data of two flavanones longitin (**170**) and isosakuranetin 7-O-rhamnoglycoside (**234**) are given in Table 12.

**Table 12. $^{1}H$ NMR data of flavanone glycosides**

| | 170 | 234 |
|---|---|---|
| H-2 | 5.44dd | 5.48dd |
| H-3a | 2.81dd | 3.20dd |
| H-3b | 2.76dd | 2.80dd |
| H-6 | 6.11d | 6.12d |
| H-8 | 6.07d | 6.15d |
| H-2' | 7.38d | 7.45d |
| H-3' | 6.95d | 6.85d |
| H-5' | 6.95d | 6.85d |
| H-6' | 7.38d | 7.45d |
| H-5OH | 12.03s | 12.02s |
| G-1 | 5.34d | 5.05d |
| Rh-1 | 4.85d | 5.20d |
| Rh-Me | 1.14d | 1.14d |
| OMe | 3.82s | 3.80s |

## *$^{13}C$ NMR Spectra*

The $^{13}C$ NMR spectral data of simple flavones and their methyl derivatives, luteolin (**6**), cirsilineol (**9**), viscidulin III (**64**), rivularin (**113**) are given in Table 13.

**Table 13. $^{13}C$ NMR Data of Some Flavones and Their Methyl Derivatives**

| | 6 | 9 | 64 | 113 |
|---|---|---|---|---|
| C-2 | 164.5s | 163.9s | 161.7s | 162.2s |
| C-3 | 103.6d | 102.9d | 111.5d | 112.1d |
| C-4 | 182.1s | 182.1s | 181.7s | 182.2s |
| C-5 | 161.9s | 151.9s | 156.2s | 156.8s |
| C-6 | 99.4d | 131.7s | 99.0d | 96.3d |
| C-7 | 165.0s | 152.5s | 157.1s | 158.4s |
| C-8 | 94.8d | 91.6d | 127.5s | 129.0s |
| C-9 | 157.9s | 158.5s | 150.3s | 150.0s |
| C-10 | 104.8s | 105.0s | 103.6s | 104.5s |
| C-1' | 122.0s | 121.3s | 114.8s | 109.8s |
| C-2' | 113.8d | 110.2d | 148.2s | 158.7s |
| C-3' | 146.2s | 147.9s | 111.1d | 102.9d |
| C-4' | 150.2s | 150.8s | 119.8d | 132.3d |
| C-5' | 116.3d | 115.7d | 142.4d | 109.3d |
| C-6' | 119.3d | 120.4d | 145.9d | 156.7s |
| OMe | - | 56.0q | 60.9q | 56.2q |
| OMe | - | 56.4q | 61.5q | 61.0q |
| OMe | - | 59.9q | - | 56.6q |

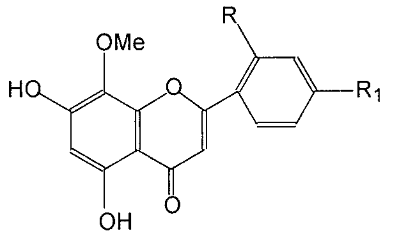
Table 14 gives the $^{13}C$ NMR data of eriodictyol (**112**), longitin (**170**), 5, 2',6'- trihydroxy - 6,7,8 - trimethoxyflavone 2'- O - β -D-glucopyranoside (**253**), 5,2',6'- trihydroxy -6,7-dimethoxyflavone 2' -O- β-D-glucopyranoside (**254**).

**Table 14. $^{13}C$ NMR of Some Flavone Glucosides and Flavanones (δ)**

| | 112 | 170 | 253 | 254 |
|---|---|---|---|---|
| C-2 | 73.7d | 78.9d | 163.2s | 163.4s |
| C-3 | 40.9t | 43.1t | 113.9d | 113.6d |
| C-4 | 195.4s | 195.4s | 184.5s | 184.1s |
| C-5 | 163.1s | 160.1s | 150.7s | 151.6s |
| C-6 | 95.5d | 97.3d | 136.5s | 130.1s |
| C-7 | 166.2s | 166.5s | 154.3s | 159.9s |
| C-8 | 94.6d | 96.0d | 133.6s | 96.8d |
| C-9 | 162.7s | 162.0s | 148.3s | 157.6s |
| C-10 | 101.4s | 104.4s | 108.4s | 105.7s |
| C-1' | 125.2s | 130.1s | 112.2s | 111.8s |
| C-2' | 112.9d | 127.7d | 157.8s | 158.0s |
| C-3' | 149.7s | 114.2d | 111.3d | 111.1d |
| C-4' | 146.1s | 160.1s | 133.7d | 133.7d |
| C-5' | 115.4d | 114.2d | 102.4d | 101.8d |
| C-6' | 116.2d | 127.7d | 158.1s | 158.2s |
| OMe | - | 55.3q | 61.5q | 57.3q |
| OMe | - | - | 62.5q | 62.3q |
| OMe | - | - | 63.1q | - |
| G-1 | - | 100.4d | 107.9d | 107.2d |
| G-2 | - | 68.8-73.4 | 74.9d | 74.5d |
| G-3 | - | " " | 78.3d | 77.7d |
| G-4 | - | " " | 71.7d | 71.0d |
| G-5 | - | " " | 78.4d | 77.0d |
| G-6 | - | 66.3t | 63.0t | 62.5t |
| Rh-1 | - | 99.8d | - | - |
| Rh-2 | - | 68.8-73.4 | - | - |
| Rh-3 | - | " " | - | - |
| Rh-4 | - | " " | - | - |
| Rh-5 | - | " " | - | - |
| Rh-6 | - | 18.6q | - | - |

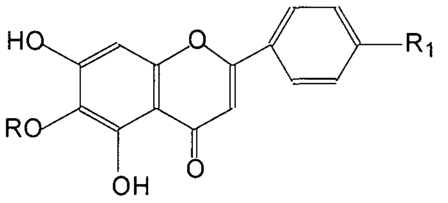
**Biological Activities of Flavonoids**

The flavonoids, obtained from a number of genera in Labiatae family have possessed pharmacological activities, among them genus *Scutellaria* is the most studied and has quite a number of activities. *Scutellaria baicalensis* is a herb investigated all around the world in detail, but especially in China and Japan. In Chinese medicine, the studies indicated that baicalin (**56**) inhibits human T-cell leukemia virus type I (HTLV-I). Baicalin produced concentration dependent inhibition of HTLV-I replication in productively infected T- and B-lympocytes [135]. Antiinflammatory activity of baicalein (**55**), was greater in the chronic inflammation model than observed in the rat carrageenan-induced paw edema $ED_{50} \geq 200.0$ mg/kg [136]. The effect of baicalein (**55**), baicalin (**56**) and wogonin (**53**) obtained from *S. baicalensis* was investigated on proliferative responses on cultured rabbit vascular smooth muscle cells. The proliferative response was determined from the up take of tritiated thymidine. In rabbit vascular smooth muscle cells, all three flavonoids dose dependently inhibited the proliferative response induced by 5% fetal calf serum, compounds **53** and **56** were less effective than baicalein (**55**) [137].

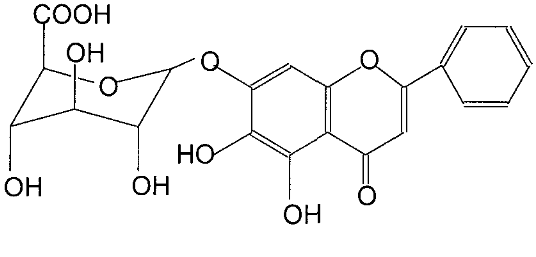



| | | |
|---|---|---|
| **53** | **R** = **R**$_1$ = H | Wogonin |
| **54** | **R** = OMe, **R**$_1$ = H | 5,7-Dihydroxy-8,2'-dimethoxyflavone |
| **255** | **R** = H, **R**$_1$ = OH | Isoscutellarein-8-methyl ether |

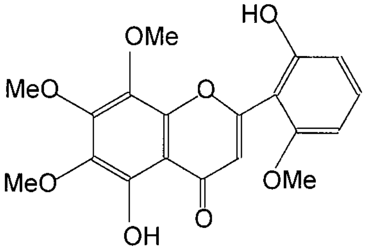
Both methanolic extract of the roots of *S .baicalensis* as well as three flavonoids **53**, **55** and **56** were evaluated for antiinflammatory action and the activatory effect on gingival fibroblasts [26].



**55** **R** = H, $\mathbf{R_1}$= H Baicalein
**57** **R** = Me, $\mathbf{R_1}$= H Oroxylin A
**72** **R** = H, $\mathbf{R_1}$ = OH Scutellarein



**56** Baicalin



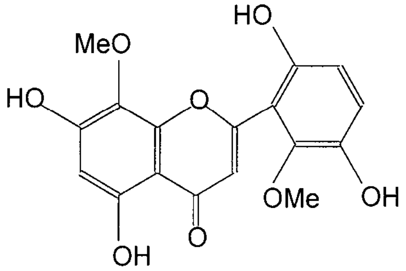
**58** Skullcapflavone II

In LPS-induced production of IL-1*β*, three flavonoids at 1 μg/ml expressed a significant (>50%) inhibitory effect, similar to that of prednisolone. Flavonoids, also inhibited IL-1*β*-induced synthesis of $PGE_2$ and $LTB_4$ considerably, although the effect of wogonin (**53**) on $LTB_4$ synthesis was marginal. The flavone isoscutellarein 8-methyl ether (**255**) obtained from the root extract of the same plant was investigated on the

single-cycle replication of mouse-adapted influenza viruses A/Guizhou/54/89 ($H_3N_2$ subtype) and B/Ibaraki/2/85 in Madin-Darby canine kidney (MDCK) cells. The agent suppressed replication of these viruses 50% at 20 μM and 90% at 40 μM [138]. Methanol extracts of the roots and the flavonoids thus obtained (baicalein and baicalin) have anti-thrombotic action. In order to classify the anti-thrombotic action of various flavonoids isolated from the above roots were examined on the t-PA and PAI-1 production by the inflammatory cytokines such as IL-1*β* and TNF-α in cultured HUVECs. In this study it was found that baicalein (**55**) dose-dependently reduced the increase of PAI-1 production of cytokines, however it didn't effect the reduction of t-PA production by IL-1*β* and TNF-α [139]. Nine flavonoids isolated from *S. baicalensis* were examined on interleukin-1*β* (IL-1*β*) and tumor necrosis factor-α (TNF-α)-induced adhesion molecular expression in cultured human umbilical vein endothelial cells (HUVECs). Among the nine flavonoids baicalein (**55**) dose-dependently inhibited IL-1*β* and TNF-α-induced endothelial leukocyte adhesion $mol^{-1}$ (ELAM-1) and intracellular adhesion $mol^{-1}$ (ICAM-1) expressions [140].

Antioxidant activity of the three flavonoids **53, 55** and **56** was studied on lipid peroxidation. Baicalin (**56**) was found the most effective [141]. As it is known α-glucosidase catalyzes the final step in the digestive process of carbohydrates and hence α-glucosidase inhibitors could retard the absorption of dietary carbohydrates to suppress postprandial hyper-glycemia. In the screening experiments for rat intestinal sucrase inhibitors in traditional Chinese herbal medicines 27 materials were tested only the methanol extract of *S. baicalensis* and *Paeonia suffriticosa* showed potent inhibitory activity against rat intestinal sucrase. The active compound of *S. baicalensis* was found to be baicalein (**55**) [142]. A benzodiazepine binding assay directed separation led to the identification of three flavones as baicalein (**55**), oroxylin A (**57**) and skullcapflavone II (**58**). Compounds **55**, **57** and **58** interacted with the benzodiazepine binding site of $GABA_A$ receptors with a $K_i$ value 13.1, 14.6 and 0.36 μmol/L respectively [27]. In a similar study using a radioreceptor binding assay to guide the isolation of four flavonoids obtained from *S. baicalensis* capable of binding to the benzodiazepine site (BZB-S) of the $GABA_A$ receptor: wogonin (**53**) ($K_i$ =2.03 – 0.24 μM), baicalin (**56**) ($K_i$=77.10–4.79 μM), baicalein (**55**) ($K_i$ =5.69-0.95 μM) and scutellarein (**72**) ($K_i$ = 12.00- 1.27 μM). Based on BZD-S binding, the order of affinity among these four compounds was

wogonin > baicalein > scutellarein > baicalin [143]. By using HBV-producing cell line (MS-$G_2$) in vitro culture system, it was found that wogonin (**53**) isolated from *S. baicalensis* suppress HBV surface antigen production (P<0.001) without evidence of cytotoxicity. By assaying the endogenous HBV DNA polymerase activity, it was found that both the relaxed circular and linear forms of HBV DNA are significantly reduced in the wogonin-treated group [144]. In a bioassay-guided fractionation of an $H_2O$ extract of the roots of *S. baicalensis* has furnished an in vitro antigenotoxic flavonoid baicalein (**55**) and 5,7,2',5'-tetrahydroxy-8,6'-dimethoxy flavone (**64**).




**64**

Baicalein exhibited a dose dependent inhibition of aflatoxin $B_1$ ($AFB_1$) and N-methyl-N'-nitro-N-nitroso guanidine mutagenicity in the *Salmonella typhimurium* bacterial mutation assay. In the chromosome aberration assay, **55**, at a concentration of 5 μM, reduced the frequency of chromosome aberration induced by $AFB_1$ but increased the clastogenic effect of $AFB_1$ at a concentration of 50 μM [145]. Flavonoids isolated from the roots of *S. baicalensis* Georgi were examined on adhesion molecule expression induced by thrombin and thrombin receptor agonist peptide (SFLLRNPNDKYEPF, TRAP) in cultured human umbilical vein endothelial cells. Thrombin and thrombin receptor agonist peptide induced endothelial leukocyte adhesion molecule-1 (ELAM-1) expression. Intercellular adhesion molecule-1 (ICAM-1) expression was also induced by thrombin, but not by TRAP. Baicalein (**55**) inhibited ELAM-1 expression induced by thrombin and thrombin agonist peptide dose-dependently, with 50% inhibitory concentrations ($IC_{50}$) of 5.53 - 1.68 μM and 2.44 - 1.08 μM respectively. Furthermore, baicalein inhibited thrombin-induced ICAM-1 expression with an $IC_{50}$ of 9.67 ‡ 1,28 μM. In

addition, baicalein inhibited the expressions of ELAM-1 and ICAM-1 stimulated by protein kinase C (PKC) activator phorbol myristate acetate (PMA) [146]. The root water extract and the single flavonoids of *S. baicalensis* were examined in liver microsomes, the aqueous extract potently inhibited aflatoxin $M_1$ ($AFM_1$), by cytochrome $P_{450}$ (CYP) 1A ½ and slightly reduced aflatoxin $B_1$ ($AFB_1$)-8,9-epoxide (AFBO) formation by $CYP_1$ A ½ , $CYP_2$ $B_1$, $CYP_2$ $C_{11}$ and $CYP_3$ A ½ in TCDD-treated rat liver microsomes. $IC_{50}$ values for $AFM_1$ and $AFB_0$ formation were 6.8 and 122.4 μg/ml respectively. Wogonin (**53**) showed the highest inhibitory activity towards $AFM_1$ formations among the isolated flavonoids [147]. Four major flavonoids of *S. baicalensis* roots showed inhibitory activity on eotaxin production at a concentration of 10 μg/ml in the order baicalein (**55**) >oroxylin A (**57**) > baicalin (**56**) > skullcapflavon II (**58**). The inhibitory effect of baicalein was expressed in a dose-dependant manner, and almost 50% inhibition was observed a 1.8μg/ml. Furthermore, baicalein prevented human eotaxoin mRNA expression in IL-4 plus TNF-α-stimulated human fibroblasts. These results help explain the efficacy of *Scutellaria* root in the treatment of bronchial asthma since it would suppress eotaxin associated recruitment of eosinophils [148]. *Scutellaria indica* which is a plant used in China under the name of Han-xincao has a folkloric use in the treatment of hemoptysis, hematemesis, and is used as anticancer agent. In vitro screening tests against L 1210 and HL60 cells showed three flavanones 2(S)-5,7-dihydroxy-8,2'-dimethoxyflavanone (**50**), 2(S)-5,7,2'-trihydroxy-8-methoxyflavanone (**51**), 2(S)-5,2',5'-trihydroxy-7,8-dimet -hoxyflavanone (**52**) and two flavones wogonin (**53**), 5,7-dihydroxy-8,2'-dimethoxyflavone (**54**). Compound **52** showed the most potent activity against L 1210 cells with an $ED_{50}$ value of 0.9 μg/ml, also it expressed a potent and wide spectrum activity against the other cell lines tested $ED_{50}$ values for HL-60 cells, K562 cells and SNU-1 cells were 0.6 μg/ml, 1.3 μg/ml and 4.7 μg/ml, respectively. The activity of the other compounds except for wogonin (**53**) was insignificant [25].

*Scutellaria rivulis* Benth is a folk medicine in Taiwan, used for the treatment of tumors, hepatitis, liver cirrhosis, jaundice. The chloroform extract of the plant demonstrated the most hepatoprotective effect of five other extracts. Consequently, three major components baicalein (**55**), baicalin (**56**) and wogonin (**53**) of this extract were further investigated for their hepatoprotective activity. This was done in vivo using three liver damage models, induced by APAP, $CCl_4$ and D-GalN in rats. The

activities of sGOT and sGPT after administration of APAP are as follows; wogonin (5, 10, 20 mg/kg) exhibited the best effect by lowering both sGOT and sGPT levels, baicalein (5, 10, 20 mg/kg) also had a moderate hepatoprotective effect, but baicalin (5, 10, 20 mg/kg) had no significant effect on sGOT activity. APAP-induced damage was more sever than the other treated groups [149].

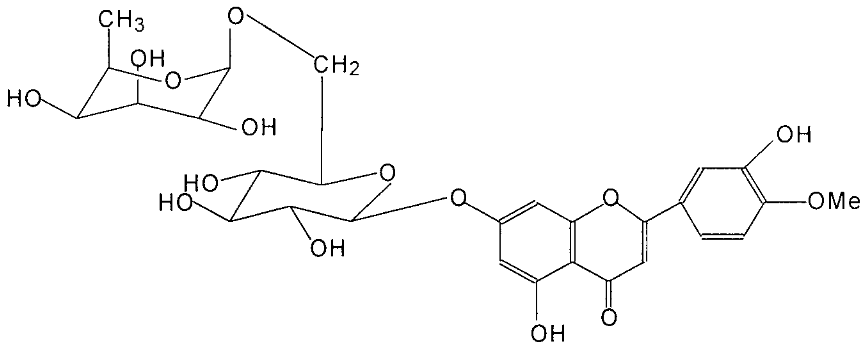
*Sideritis mugronensis* an endemic plant of eastern Spain and is used in folk medicine as anti-inflammatory and antirheumatic agent. The active principle was found to be hypolaetin 8-glucoside (**140**) [58].

*Marrubium alysson* L. has a wide reputation in Egypt as a diuretic and a remedy for asthma. Apigenin (**18**) and apigenin 7-glucoside (**19**) and its arabinoside (**196**) were isolated together with other some compounds [90].

*Sideritis javalambrensis* Pau is an endemic species growing in Sierra javalambre (Teruel, Spain). The plant is known to possess anti-inflammatory activity. In a screening test a group of flavonoid aglycones were isolated, cirsiliol (**116**), cirsimaritin (**2**), 8-methoxycirsilineol (**153**), sideritoflavone (**154**) and xanthomicrol (**132**) and their anti-inflammatory properties were studied. As a result 8-methoxycirsilineol (**153**) and cirsimaritin (**2**) were found to inhibit microsomal lipid peroxidation. Also the plant has yielded glycosides among them hypolaetin 8-glucoside (**140**) exhibit a high potency as antiperoxidative agent [67]. Another *Sideritis* species *S. leucantha* Cav. from Spain also exhibited anti-inflammatory activity. A flavone obtained from this plant 5,3',4'-trihydroxy-6,7,8-trimethoxy (**256**) was found active in Carrageenan paw oedema test [150].

A *Satureja* species *S. obovata* Lag. sups. *obovata* var. *valentina* is an eastern Spanish plant and used as a relaxant on vascular and intestinal smooth muscle cells. The plant has yielded apigenin (**18**), genkwanin (**16**), luteolin (**6**), cirsimaritin (**2**), chrysoeriol (**17**), xanthomicrol (**132**), diosmetin (**10**), luteolin 7-*O*-$\beta$-D-glucoside (**7**), luteolin 7-*O*-$\beta$-D-rutinoside (**183**), luteolin 3'-*O*-$\beta$-D-glucoside (**257**), and vicenin-2 (**44**). Recently S-naringenin (**175**) and S-eriodictyol (**112**) were also isolated. The tests were carried out on isolated rat aorta, contraction was induced by KCl (80 mM) and noradrenaline (NA) ($10^{-6}$ M). The sustained contraction was relaxed by luteolin (98.7 - 0.7% ; 40.30 - 2.4%), naringenin (12.41 - 18%; 3.05 - 0.3%) and eriodictyol (67.48 - 3.5%; 17.93 - 2.1%) each at the dose $5x10^{-5}$ M, respectively [131]. Antimicrobial activity of the flavonoid of the *Satureja parvifolia* was established, together with some other plants on *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC

27853), *Klebsiella pneumonia (*ATCC 4352), *Bacillus subtilus (*BSGC $A_1$), *Stahylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228) [151]. Flavonoids obtained from *Salvia palaestina* were tested on standard bacteria cirsimaritin (**2**) and 6,7,3',4'-tetra methoxyflavone exhibited activity [12]. Two flavonoids vicenin-2 (**44**) and orientin (**135**) isolated from the leaves of *Ocimum sanctum* for testing their radioprotective effect in mice. Both compounds provided protection against death from gastrointestinal Gy gamma radiation, the optimum drug dose for protection was 50mu g/kg body weight. Maximum protection was obtained when either compound was injected i.p. 30 min. before irradiation [53]. *Mentha spicata* L. var. *crispa* Benth. is cultivated as an aromatic plant when its methanolic extract was suspended in water and successively partitioned with hexane, ethyl acetate and in 1-butanol. Each fraction was bioassayed for inhibitory activity of exocytes of rat basophyls caused by antigen-induced stimulation. This assay is used to detect promising anti-allergic reagents for type I. Significant activity was observed for the ethyl acetate fraction and four flavonoids 5-desmethoxynobiletin (**147**), 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone (**161**), thymonin (**162**) and sideritoflavone (**154**) were isolated from this fraction. [73]. A group of flavonoids obtained from *Mentha piperita* L. was tested for their antiallergic activity, only luteolin 7-rutinoside (**183**) inhibited the histamine release. [152].

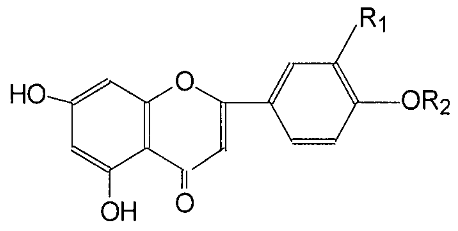



**228** Diosmin

Diosmin (**228**), a flavone glycoside obtained from *Hyssopus officinalis* L. has shown considerable pharmacological potential in the improvement of

vascular resistance; as an antioxidant; it has anti-lipid peroxidation effect; also effective in the treatment of hemorrhoidal disease, as a powerful agent against inflammatory disorders, and as an inhibitor of prostaglandin and thromboxane synthesis, increases capillary resistance [117].

In Uzbekistan Republic *Phlomis spinidens* Nevski was used as a traditional medicine in the treatment of allergy. Two new bisglycosides were isolated and termed as phlomisflavosides A (**190**) and phlomisflavosides B (**191**) together with known compounds astragalin (**127**) and isoquercitrin (**24**) [88].




| | | |
|---|---|---|
| Phlomisflavosides A | **190** | $\mathbf{R_1}$ = OH, $\mathbf{R_2}$ = Apiofuranosyl |
| Phlomisflavosides B | **191** | $\mathbf{R_1}$ = H, $\mathbf{R_2}$ = Apiofuranosyl |

*Ajuga iva* (L.) Schreber has examined for its hypoglycaemic effect in normal and streptozotocin-induced rats, and a significant decrease was observed in plasma glucose levels. Among other compounds, flavonoids were the major constituents of the aqueous extract of this plant [153].

## REFERENCES

[1] Swain, T. In *Flavonoids*; Harborne, J.B., Mabry, T.J. and Mabry, H., Ed.; Chapman and Hall: London, **1975**; pp.1097-1129.

[2] Mabry, T.J. and Ulubelen, A.; *J. Agric.and Food Chem.*, **1980**, 28, 188-196.

[3] Seikel, M.K. In *The Chemistry of Flavonoid Compounds*; Geissman, T.A., Ed.; Pergamon Press: Oxford, **1962**; pp. 34-69.

[4] Mabry, T.J., Markham, K.R. and Thomas, M.B. *The Systematic*

*Identification of Flavonoids*, Springer-Verlag, Berlin, **1970**, pp.16-22.
[5] Markham, K.R. and Chari, V.M. In *The Flavonoids*; Harborne, J.B., Mabry, T.J., Ed.; Chapman and Hall: London, **1982**; pp. 19-134.
[6] Mabry, T.J. and Ulubelen, A. *Biochemical Applications of Mass Spectrometry, First Supplementary*; Ed. G.R. Waller and O.C. Dermer, **1980**, pp.1132-1156.
[7] Davis, P.H., *Flora of Turkey and East Aegean Islands*; Edinburg Press, Edinburg, **1982**, Vol. 7, pp. 400.
[8] Ulubelen, A.; Ozturk, S.; Isıldatıcı, S.; *J. Pharm. Sci.*, **1968**, 57, 1037- 1038.
[9] Ulubelen, A.; Miski, M.; Neuman, P.; Mabry, T.J.; *J. Nat. Prod.*, **1979**, 42, 261-262.
[10] Ulubelen, A.; Miski, M.; Mabry, T.J.; *J. Nat. Prod.*, **1981**, 44, 586-587.
[11] Brieskorn, C.H., Kapadia, Z.; *Planta Medica*, **1979**, 35, 376-378.
[12] Miski, M.; Ulubelen, A.; Johansson, C.; Mabry, T.J.; *J. Nat. Prod.*, **1983**, 46, 874-875.
[13] Ulubelen, A.; Topcu, G.; *J. Nat. Prod.*, **1984**, 47, 1068.
[14] Ulubelen, A.; Topcu, G.; Eris, C.; Sonmez, U.; Kartal, M.; Kurucu, S.; Bozok-Johansson, C.; *Phytochemistry*, **1994**, 36, 971-974.
[15] Ulubelen, A.; Topcu, G.; Sonmez, U.; Eris, C.;*Phytochemistry*,**1994**, 35, 1065-1067.
[16] Ulubelen, A.; Tuzlacı, E.; *Fitoterapia*, **1990**, 61, 185.
[17] Gokdil, G.; Topcu, G.; Sonmez, U.; Ulubelen, A.; *Phytochemistry*, **1997**, 46, 799-800.
[18] Bisio, A.; Romussi, G.; Ciarallo, G.; De Tomassi, N.; *Pharmazie*, **1997**, 52, 330-331.
[19] Zahid, M.; Ishrud, O.; Pan, Y.J.; Asim, M.; Riaz, M.; Ahmad, V.U.; *Carbohydrate Research*, **2002**, 337, 403-407.
[20] Veitch, N. C.; Grayer, R. J.; Irwin, J. L.; Takeda, K.; *Phytochemistry*, **1998**, 48, 389-393.
[21] Areias, F.; Valentao, P.; Andrade, P.B.; Ferreres, F.; Seabra, R.M.; *J. Agricul. and Food Chem.*, **2000**, 48, 6081-6084.
[22] Lu, Y.; Foo, L.Y.; *Phytochemistry*, **2000**, 55, 263-267.
[23] Weng, X.C.; Wang, W.; *Food Chemistry*, **2000**, 71, 489-493.
[24] Ahmad, V.U.; Ali, Z.; Zahid, M.; Alam, N.; Saba, N.; Khan, T.;

Qaisar, M.; Nisar, M.; *Fitoterapia*, **2000**, 71, 84-85.
[25] Bae, K.H.; Min, B.S.; Park, K.L.; Ahn, B.Z.; *Planta Medica*, **1994**, 60, 280-281.
[26] Chung, C.P.; Park, J.B.; Bae, K.H.; *Planta Medica*, **1995**, 61, 150-153.
[27] Liao, J.F.; Wang, H.H.; Chen, M.C.; Chen, C.C.; Chen, C.F.; *Planta Medica*, **1998**, 64, 571-572.
[28] Zhang, Y.Y.; Guo, Y.Z.; Ageta, H.; Harigaya, Y.; Onda, M.; Hashimoto, K.; Ikeya, Y.; Okada, M.; Maruno, M.; *Planta Medica*, **1997**, 63, 536-539.
[29] Yuldashev, M.P.; Karimov, A.; *Chem. Nat. Compds.*, **2001**, 37, 431-433.
[30] Yuldashev, M.P.; *Chem. Nat. Compds.*, **2001**, 37, 428-430.
[31] Ersoz, T.; Harput, U.S.; Saracoglu, I.; Calıs, I.; Ogihara, Y.; *Turkish J. of Chem.*, **2002**, 26, 581-588.
[32] Danikeeva, M.F.; Litvinenko, V.I.; Borodin, L.I.; *Khim.Prir.Soedin.*, **1970**, 6, 534-539; CA 74, 39163 h (**1971**).
[33] Litvinenko, V.I.; Danikeeva, M.F.; *Khim.Prir.Soedin.*, **1971**, 7, 375-376; CA 75, 115877 g, **1971**.
[34] Tomimori, T.; Miyaichi, Y.; Imoto, Y.; Kizu, H.; Suzuki, C.; *Yakugaku Zasshi*, **1984**, 104, 529-534; CA 101, 167117 j, **1984**.
[35] Tomimori, T.; Miyaichi, Y.; Imoto, Y.; Kizu, H.; Tanabe, Y.; *Yakugaku Zasshi*, **1984**, 104, 524-528; CA 101, 167116 i, **1984**.
[36] Beshko, N.P.; Gella, E.V.; Litvinenko, V.I.; Kovalev, I.P.; Gordienko, V.G.; *Khim.Prir.Soedin.*, **1975**, 11, 514; CA 84, 27991 v, **1976**.
[37] Nicollier, G.F.; Thompson, A.C.; Salin, M.L.; *J. Agric. and Food Chem.*, **1981**, 29, 1179-1181.
[38] Takagi, S.; Yamaki, M.; Inoue, K.; *Phytochemistry*, **1981**, 20, 2443-2444.
[39] Tomimori, T.; Miyaichi, Y.; Imoto, Y.; Kizu, H.; Namba, T.; *Chem. Pharm. Bull.*, **1985**, 33, 4457-4463.
[40] Miyaichi, Y.; Imoto, Y.; Tomimori, T.; Lin, C.C.; *Chem. Pharm. Bull.*, **1987**, 35, 3720-3725.
[41] Miyaichi, Y.; Imoto, Y.; Tomimori, T.; Namba, T.; *Chem. Pharm. Bull.*, **1988**, 36, 2371-2376.
[42] Kikuchi, Y.; Miyaichi, Y.; Tomimori, T.; *Chem. Pharm. Bull.*, **1991**,

39, 1466-1472.
[43] Zhang, Y.Y.; Guo, Y.Z.; Onda, M.; Hashimoto, K.; Ikeya, Y.; Okada, M.; Maruno, M.; *Phytochemistry*, **1994**, 35, 511-514.
[44] Ishimaru, K.; Nishikawa, K.; Omoto, T.; Asai, I.; Yoshihara, K.; Shimomura, K.; *Phytochemistry*, **1995**, 40, 279-281.
[45] Kisiel, W.; Stojakowska, A.; Piozzi, F.; Rosselli, S.; *Acta Societatis Botanicorum Poloniae*, **2001**, 70, 199-201.
[46] Kawashty, S.A.; Gamal El-Din, E.M.; Saleh, N.A.M.; *Biochemical Systematics and Ecology*, **1999**, 27, 657-660.
[47] Harborne, J.B.; Tomas-Barberan, F.A.; Williams, C.A.; Gil, I.; *Phytochemistry*, **1986**, 25, 2811-2816.
[48] Grayer, R.J.; Kite, G.C.; Veitch, N.C.; Eckert, M.R.; Marin, P.D.; Senanayake, P.; Paton, A.J.; *Biochemical Systematics and Ecology*, **2002**, 30, 327-342.
[49] Grayer, R.J.; Veitch, N.C.; Kite, G.C.; Price, A.M.; Kokubun, T.; *Phytochemistry*, **2001**, 56, 559-567.
[50] Xaasan, C.C.; Ciilmi, C.X.; Faarax, M.X.; Passannanti, S.; Piozzi, F.; Paternostro, M.; *Phytochemistry*, **1980**, 19, 2229-2230.
[51] Grayer, R.J.; Veitch, N.C.; *Phytochemistry*, **1998**, 47, 779-782.
[52] Grayer, R.J.; Bryan, S.E.; Veitch, N.C.; Goldstone, F.J.; Paton, A.; Wollenweber, E.; *Phytochemistry*, **1996**, 43, 1041-1047.
[53] Devi, P.U.; Ganasoundari, A.; Rao, B.S.S.; Srinivasan, K.K.; *Radiation Research*, **1999**, 151, 74-78.
[54] Grayer, R.J.; Kite, G.C.; Abou-Zaid, M.; Archer, C.J.; *Phytochemical Analysis*, **2000**, 11, 257-267.
[55] Tomasbarberan, F.A.; Gil, M.I.; Ferreres, F.; Rivera, D.; Obon, C.; Tomaslorente, F.; *Biochemical Systematics and Ecology*, **1993**, 21, 487-497.
[56] Kokkalou, E.; Gabrieli, C.; *Pharmazie*, **1997**, 52, 322-324.
[57] Rodriguez-Lyon, M.L.; Diaz-Lanza, A.M.; Bernabe, M.; Villaescusa-Castillo, L.; *Magnetic Resonance in Chemistry*, **2000**, 38, 684-687.
[58] Villar, A.; Gasco, M.A.; Alcaraz, M.J.; Manez, S.; Cortes, D.; *Planta Medica*, **1985**, 1, 70.
[59] Tomas, F.; Voirin, B.; Barberan, F.A.T.; Lebreton, P.; *Phytochemistry*, **1985**, 24, 1617-1618.
[60] Palomino, O.M.; Söllhuber, M.; Carretero, E.; Villar, A.;

*Phytochemistry*, **1996**, 42, 101-102.
[61] Venturella, P.; Bellino, A.; Marino, M.L.; *Phytochemistry*, **1995**, 38, 527-530.
[62] Barberan, F.A.T.; Tomas, F.; Ferreres, F.; *Phytochemistry*, **1984**, 23, 2112-2113.
[63] Gabrieli, C.; Kokkalou, E.; *Phytochemistry*, **1990**, 29, 681-683.
[64] Rodriguez, B.; *Phytochemistry*, **1977**, 16, 800-801.
[65] Gonzalez, A.G.; Fraga, B.M.; Hernandez, M.G.; Larruga, F.; Luis, J.G.; Ravelo, A.G.; *Lloydia*, **1978**, 41, 279-280.
[66] Fernandez, C.; Fraga, B.M.; Hernandez, M.G.; Arteaga, J.M.; *J. Nat. Prod.*, **1988**, 51, 591-593.
[67] Rios, J.L.; Manez, S.; Paya, M.; Alcaraz, M.J.; *Phytochemistry*, **1992**, 31, 1947-1950.
[68] Tomas, F.; Ferreres, F.; *Phytochemistry*, **1980**, 19, 2039-2040.
[69] Tomas, F.; Ferreres, F.; Guirado, A.; *Phytochemistry*, **1979**, 18, 185-186.
[70] Barberan, F.A.T.; Nunez, J.M.; Tomas, F.; *Phytochemistry*, **1985**, 24, 1285-1288.
[71] Al-Hazimi; Hassan, M.G.; *Orient. J. Chem.*, **1988**, 4, 281-285; CA 110, 92048 n, **1989**.
[72] Zaidi, F.; Voirin, B.; Jay, M.; Viricel, M.R.; *Phytochemistry*, **1988**, 48, 991-994.
[73] Yamamura, S.; Ozawa, K.; Ohtani, K.; Kasai, R.; Yamasaki, K.; *Phytochemistry*, **1998**, 48, 131-135.
[74] Sharaf, M.; El-Ansari, M.A.; Saleh, N.A.M.; *Fitoterapia*, **1999**, 70, 478-483.
[75] Ghoulami, S.; Il Idrissi, A.; Fkih-Tetouani, S.; *Fitoterapia*, **2001**, 72, 596-598.
[76] Ali, M.S.; Saleem, M.; Ahmad, W.; Parvez, M.; Yamdagni, R.; *Phytochemistry*, **2002**, 59, 889-895.
[77] El-Desoky, S.K.; El-Ansari, M.A.; El-Negoumy, S.I.; *Fitoterapia*, **2001**, 72, 532-537.
[78] Barberan, F.A.T.; Hernandez, L.; Ferreres, F.; Tomas, F.; *Planta Medica*, **1985**, (5), 452-454.
[79] Corticchiato, M.; Bernardini, A.; Costa, J.; Bayet, C.; Saunois, A.; Voirin, B.; *Phytochemistry*, **1995**, 40, 115-120.
[80] Miura, K.; Nakatani, N.; *Agric.Biol.Chem.*, **1989**, 53, 3043-3045.

[81] Washington, J.S.; Saxena, V.K.; *J.Ind.Chem.(India)*, **1985**, 57, 153-155; CA 104, 145503 s, **1986**.
[82] Van den Brocke, C.O.; *Verh.-K. Acad. Genees kd. Blg.*, **1983**, 45, 381-402; CA 100, 185625 g, **1984**.
[83] Ferreres, F.; Tomas, F.; Barberan, F.A.T.; Hernandez, L.; *Plant. Med.Phytother.*, **1985**, 19, 89-97.
[84] Kumar, R.; Bhan, S.; Kalla, A.K.; Dhar, K.L.; *Phytochemistry*, **1985**, 24, 1124-1125.
[85] El-Negoumy, S.I.; Abdalla, M.F.; Saleh, N.A.M.; *Phytochemistry*, **1986**, 25, 772-774.
[86] Tomas, F.; Nieto, J.L.; Barberan, F.A.T.; Ferreres, F.; *Phytochemistry*, **1986**, 25, 1253-1254.
[87] Bucar, F.; Ninov, S.; Ionkova, I.; Kartnig, Th.; Schubert-Zsilavecz, M.; Asenov, I.; Konuklugil, B.; *Phytochemistry*, **1998**, 48, 573-575.
[88] Takeda, Y.; Isai, N.; Masuda, T.; Honda, G.; Takaishi, Y.; Ito, M.; Otsuka, H.; Ashurmetov, O.A.; Khodzhimatov, O.K.; *Chem.Pharm. Bull.*, **2001**, 49, 1039-1041.
[89] Buddrus, J.; Linscheid, M.; *Phytochemistry*, **1989**, 28, 3201-3206.
[90] Saleh, M.R.I.; Sarg, T.M.; Metwally, A.M.; Rakha, A.A.; *Planta Medica*, **1981,** 41, 202-203.
[91] Kartnig, Th.; Bucar, F.; Neuhold, S.; *Planta Medica*, **1993**, 59, 563-564.
[92] Bucar, F.; Kartnig, Th.; *Planta Medica*, **1995**, 61, 378-380.
[93] Kartnig, Th.; Bucar, F.; *Planta Medica*, **1995**, 61, 392.
[94] Bucar, F.; Kartnig, Th.; Paschek, G.; Winkler, E.; Schubert-Zsilavecz, M.; *Planta Medica*, **1995**, 61, 489.
[95] Winterhoff, H.; Gumbinger, H.G.; Sourgens, H.; *Planta Medica*, **1988**, (2), 101-106.
[96] Krishnaswamy, N.R.; Seshadri, T.R.; Tahir, P.J.; *Ind. J. Chem.*, **1968**, 6, 676-677; CA 70, 58224 n, **1969**.
[97] Tomasbarberan, F.A.; Gil, M.I.; Ivancheva, S.; Tomaslorente, F.; *Biochemical Systematics and Ecology*, **1992**, 20, 589-590.
[98] Bertrand, M.C.; Tillequin, F.; Bailleul, F.; *Biochemical Systematics and Ecology*, **2000**, 28, 1031-1033.
[99] Darbour, N.; Baltassat, F.; Raynoud, J.; *Pharmazie*, **1986**, 41, 605-608.
[100] Citoglu, G.; Tanker, M.; Sever, B.; *Pharmaceutical Biology*, **1999**,

37, 158-160.
[101] Vekiari, S.A.; Oreopoulou, V.; Tzia, C.; Thomopoulos, C.D.; *J. Am. Oil Chem. Soc.*, **1993**, 70, 483-487.
[102] Zheng, S.Z.; Wang, X.X.; Gao, L.M.; Shen, X.W., Liu, Z.L.; *Ind. J.Chem. Sec . B - Org. Chem. Including Medicinal Chemistry*, **1997**, 36, 104-106.
[103] Bellakhdar, J.; Passannanti, S.; Paternostro, M.P.; Piozzi, F.; *Planta Medica*, **1988**, 54, 94.
[104] Bosabalidis, A.; Gabrieli, C.; Niopas, I.; *Phytochemistry*, **1998**, 49, 1549-1553.
[105] Kulevanova, S.; Stefova, M.; Stefkov, G.; Stafilov, T.; *J. Liq. Chro.and Rel. Tech.*, **2001**, 24, 589-600.
[106] Skaltsa, H.; Bermejo, P.; Lazari, D.; Silvan, A.M.; Skaltsounis,A.L.; Sanz, A.; Abad, M.J.; *Biol. Pharm. Bull.*, **2000**, 23, 47-53.
[107] Kotsos, M.; Aligiannis, N.; Mitaku, S.; Skaltsounis, A.L.; Charvala, C.; *Nat. Prod. Lett.*, **2001**, 15, 377-386.
[108] Sharaf, M.; *Fitoterapia*, **1998**, 69, 355-357.
[109] El-Ansari, M.A.; Barron, D.; Abdalla, M.F.; Saleh, N.A.M.; Le Quéré, J.L., *Phytochemistry*, **1991**, 30, 1169-1173.
[110] El-Ansari, M.A.; Nawwar, M.A.; Saleh, N.A.M.; *Phytochemistry*, **1995**, 40, 1543-1548.
[111] Lenherr, A.; Lahloub, M.F.; Sticher, O.; *Phytochemistry*, **1984**, 23, 2343-2345.
[112] Lenherr, A.; Mabry, T.J.; *Phytochemistry*, **1987**, 26, 1185-1188.
[113] Barberan, F.A.T.; Hernandez, L.; Tomas, F.; *Phytochemistry*, **1986**, 25, 561-562.
[114] Miski, M.; Ulubelen, A.; Mabry, T.J.; *Phytochemistry*, **1983**, 22, 2093-2094.
[115] Prokopenko, S.A.; *Khim. Prir.Soedin.*, **1986**, 514; CA 105, 222793 m, **1986**.
[116] El-Garf, I.; Grayer, R.J.; Kite, G.C.; Veitch, N.C.; *Biochem. System. and Eco.*, **1999**, 27, 843-846.
[117] Marin, F.R., Ortuno, A.; Benavente-Garcia, O.; Del Rio, J.A.; *Planta Medica*, **1998**, 64, 181-182.
[118] Budzianowski, J.; Skrzypczak, L.; *Phytochemistry*, **1995**, 38, 997-1001.

[119] Bankova, V.; Koeva-Todorovska, J.; Stambolijska, T.; Ignatova-Groceva, M.D., Todorova, D.; Popov, S.; *Zeitschrift für Naturforschung C*, **1999**, 54, 876-880.
[120] Marin, P.D.; Grayer, R.J.; Veitch, N.C.; Kite, G.C.; Harborne, J.B.; *Phytochemistry*, **2001**, 58, 943-947.
[121] Dardass, A.K.; Firdous, S.; Ali, Z.; Ahmad, V.U.; *Zeitschrift für Naturforschung B*, **1999**, 54, 569-571.
[122] Tomasbarberan, F.A.; Gil, M.I.; Ferreres, F.; Tomaslorente, F.; *Phytochemistry*, **1991**, 30, 3311-3314.
[123] Dmitruk, I.S.; Dmitruk, S.E.; Berezovskaya, T.P.; Pirshchep, T.P.; *Khim.Prir.Soedin.*, **1987**, 449-450; CA 107, 151266 v, **1987**.
[124] Doganca, S.; Ulubelen, A.; Tuzlacı, E.; *Phytochemistry*, **1989**, 28, 3561-3562.
[125] Shamyrina, A.A.; Peshkova, V.A.; Shergina, N.I.; *Khim.Prir. Soedin.*; **1975**, 11, 255; CA 83, 111116 a, **1975**.
[126] Kartnig, Th.; Gruber, A.; Menzinger, S.; *J.Nat.Prod.*, **1985**, 48, 494.
[127] Tomasbarberan, F.A.; Krestovskaya, T.; Gil, M.I.; *Biochemical Systematics and Ecology*, **1993**, 21, 531-532.
[128] Heitz, A.; Carnat, A.; Fraisse, D.; Carnat, A.P.; Lamaison, J.L.; *Fitoterapia*, **2000**, 71, 201-202.
[129] Mulkens, A.; Kapetanidis, I.; *Pharm. Acta Helv.*, **1987**, 62, 19-22.
[130] Sanchez de Rojas, V.R.; Somoza, B.; Ortega, T.; Villar, A.M.; *Planta Medica*, **1996**, 62, 554-555.
[131] Sanchez de Rojas, V.R.; Somoza, B.; Ortega, T.; Villar, A.M.; *Planta Medica*, **1996**, 62, 272-274.
[132] Kokkalou, E.; Kapetanidis, I.; *Pharm. Acta Helv.*, **1988**, 63, 170-173.
[133] Lu, J.S.; Shen, T.; Guo, Z.; Shen, X.W.; Zheng, S.Z.; *Acta Botanica Sinica*, **2001**, 43, 545-550.
[134] Zheng, S.Z.; Kang, S.H.; Shen, Y.W.; Sun, L.P.; *Planta Medica*, **1999**, 65, 173-175.
[135] Baylor, N.W.; Fu, T.; Yan, Y.D.; Ruscetti, F.W.; *J. Infect. Dis.*, **1992**, 165, 433-437; CA 116, 187547 n, **1992**.
[136] Butenko, I.G.; Gladtchenko, S.V.; Galushko, S.V.; *Agents Actions*, **1993**, 39, C 49-51; CA 120, 277s, **1994**.
[137] Huang, H.C.; Wang, H.R.; Hsieh, L.M.; *Eur. J. Pharmacol.*, **1994**, 251, 91-93.

[138] Nagai, T.; Suzuki, Y.; Tomimori, T.; Yamada, H.; *Biol. Pharm. Bull*, **1995**, 18, 295-299; CA 122, 230220 p, **1995**.

[139] Kimura, Y.; Okuda, H.; Yokoi (Hayashi), K.; Matsushita, N.; *Phytotherapy Research*, **1997**, 11, 363-367.

[140] Kimura, Y.; Matsushita, N.; Hiromichi, O.; *J. Ethnopharmacol.*, **1997**, 57, 63-67.

[141] Gabrielska, J.; Oszmianski, J.; Zylka, R.; Komorowska, M.; *Z. Naturforsch. C. Biosci.*, **1997**, 52, 817-823.

[142] Nishioka, T.; Kawabata, J.; Aoyama, Y.; *J. Nat. Prod.*, **1998**, 61, 1413-1415.

[143] Hui, K.M.; Wang, X.H.; Xue, H.; *Planta Medica*, **2000**, 66, 91-93.

[144] Huang, R.L.; Chen, C.C.; Huang, H.L.; Chang, C.G.; Chen, C.F.; Chang, C.; Hsieh, M.T.; *Planta Medica*, **2000**, 66, 694-698.

[145] Lee, B.H.; Lee, S.J.; Kang, T.H.; Kim, D.H.; Sohn, D.H.; Ko, G.I.; Kim, Y.C.; *Planta Medica*, **2000**, 66, 70-71.

[146] Kimura, Y.; Matsushita, N.; Yokoi-Hayashi, K.; Okuda, H.; *Planta Medica*, **2001**, 67, 331-334.

[147] Kim, B.R.; Kim, D.H.; Park, R.; Kwon, K.B.; Ryu, D.G.; Kim, Y.C.; Kim, N.Y.; Jeong, S.; Kang, B.K.; Kim, K.S.; *Planta Medica*, **2001**, 67, 396-399.

[148] Nakajima, T.; Imanishi, M.; Yamamoto, K.; Cyong, J.C.; Hirai, K.; *Planta Medica*, **2001**, 67, 132-135.

[149] Lin, C.C.; Shieh, D.E.; *Phytotherapy Research*, **1996**, 10, 651-654.

[150] Jimenez, M.J.; Alcaraz, M.J.; Ferrandiz, M.L.; Villar, A.; *Planta Medica*, **1986**, (6), 541.

[151] Hernandez, N.E.; Tereschuk, M.L.; Abdala, L.R.; *J.Ethnopharmacology.*, **2000**, 73, 317-322.

[152] Inoue, T.; Sugimoto, Y.; Masuda, H.; Kamei, C.; *Biol. Pharm. Bull.*, **2002**, 25, 256-259.

[153] El Hilaly, J.; Lyoussi, B.; *J.Ethnopharmacology.*, **2002**, 80, 109-113.

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# BIOLOGICAL ACTIVITY OF QUINONES

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**ABSTRACT:** Quinones constitute a structurally diverse class of phenolic compounds with a wide range of pharmacologial properties, which are the basis for different applications in the broad field of pharmacy and medicine. In traditional medicine all over the world, plants which are rich in quinones are used for the treatment of a variety of diseases. Besides the classical applications of these plants in industry (dyestuffs) and pharmaceutical (laxatives) practice, the relatively new field of biologically active quinones will be discussed. This review gives an account of the work done on naturally occurring bioactive quinones from 1992 to the present date. The biological activity detected in quinones from natural and synthetic sources has been discussed in relation to chemical structure under the respective titles.

## INTRODUCTION

Long before anything was known of their chemistry, rhubarb, aloes, senna and cascara were recognized as forming a natural group of laxative drugs. Moreover certain vegetable and animal dyestuffs such as madder and cochineal were of great economic importance before the introduction of synthetic dyestuffs. Later the chemical similarity of these laxative drugs and dyestuffs became apparent. The establishment of the common basic structural pattern (a *para*- or an *ortho*- substituted dione in conjugation with the doble bonds of a benzene or condensed aromatic ring system) provided the basis for the systematic chemical recognition and identification of quinones from natural sources. Knowledge of comparative taxonomy, biological actions and ecological interactions has greatly contributed to the discovery of new quinones.

In traditional medicine all over the world, plants which are rich in quinones are used for the treatment of a variety of diseases. The biological

activity of quinones has prompted several pharmacological studies in order to ascertain their possible use in medicine. This review gives an account of the work done on bioactive quinones from 1992 to the present date. The biological activity detected in quinones from natural and synthetic sources has been discussed in relation to chemical structure under the respective titles. Information on novel quinones and new plant sources of quinones is also updated. A separate section deals with quinone production in plant cell and tissue cultures, since cultured plant cells have proven to be a very useful tool in biosynthesis studies. Cell cultures also occasionally accumulate new metabolites which are not found in the parent plants.

## OCCURRENCE AND STRUCTURAL CHARACTERISTICS

Quinones are found in bacteria, fungi, lichens, Gymnosperms and Angiosperms [1-7]. In the animal kingdom, quinones occur in echinoderms, e.g. isoprenoid quinones in sea urchins [8] and arthropods, e.g. anthraquinones in insects such as cochineal [9,10]. Plastoquinones, α-tocopherolquinones and phylloquinone are primary metabolites, probably present in all photosynthesising tissues, whereas ubiquinones have been found in most plants, and generally in animals. The majority of quinones found in plants are relatively simple benzoquinones, naphthoquinones or anthraquinones, although less common skeletal structures are also found to occur, such as terpenoid quinones and higher polycyclic quinones. Most quinones found in nature are *p*-quinones, but *o*-quinones also exist.

In higher plants, anthraquinones are found in the Rubiaceae, Leguminoseae, Rhamnaceae, Polygonaceae, Liliaceae and Scrophulariaceae. Most naphthoquinones occur in Bignonaceae, Verbenaceae, Juglandaceae, Plumbaginaceae, Borraginaceae, Lythraceae, Balsaminaceae, Ebenaceae and Droseraceae. Myrsinaceae and Borraginaceae are some families in which benzoquinones are found to accumulate.

## LAXATIVE ACTIVITY

The anthranoid compounds, which can be chemically described as dihydroxy-anthraquinones, -dianthrones and –anthrones, possess a laxative

effect. As these substances are the constituents of some plants and their extracts (aloe, cascara, frangula, rhubarb and senna) they are often referred to as vegetable laxatives. Because of their chemical structure, they are carried unabsorbed to the large bowel, where metabolism to active aglycones takes place. The active aglycon is released by bacterial hydrolysis of the sugar. The intestinal bacterial flora also accounts for the reduction of anthraquinone aglycons to the corresponding anthrones [11-13]. These aglycones exert their laxative effect by damaging epithelial cells, which leads directly and indirectly to changes in absorption, secretion and motility. After adsorption, the anthranoids are transformed mainly to their corresponding glucuronide and sulfate derivatives, which appear in urine and bile. However, chronic abuse of anthranoid laxatives has recently been associated with an increased risk of colorectal carcinoma, as we will see.

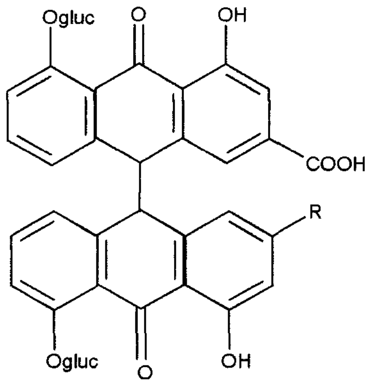


**Fig. (1).** Structure of sennosides

Several studies have been conducted in order to establish and better understand the mode of action of anthranoid laxatives. For example, Lemli [14] presented a review of the study of the mode of action of sennosides, Fig. **(1)** the active constituents of the senna drug. An interaction between rhein-anthrone, the active metabolite of sennosides, and the immune cells of the colon is suggested as a base for laxative activity. Several studies aimed to

explore the mechanism involved in the synergistic purgative actions of rhein-anthrone and aloe-emodin-anthrone, another active metabolite of sennosides.

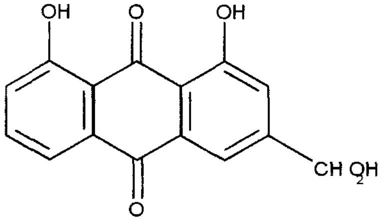


**Fig. (2).** Structure of aloe-emodin

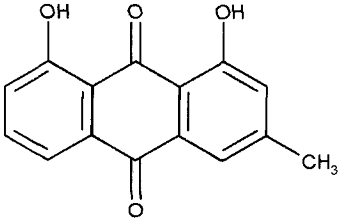


**Fig. (3).** Structure of crisophanol

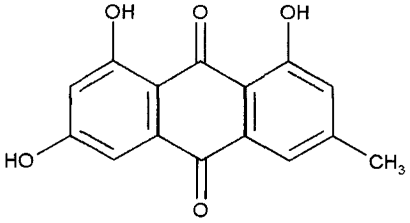


**Fig. (4).** Structure of emodin

This synergistic effect in mice results from synergistic stimulation of large intestinal transit and large intestinal water secretion [15,16]. Recently, several investigations have been performed to determine whether intracaecally administered rhein-anthrone and anthraquinones such as aloe-emodin, Fig.

(2), chrysophanol, Fig. (3), emodin, Fig. (4) or rhein, Fig. (5) synergistically enhance purgative action, as has been observed for rhein-anthrone and aloe-emodin-anthrone. These results confirmed that rhein-anthrone and aloe-emodin synergistically exert a purgative action [17].

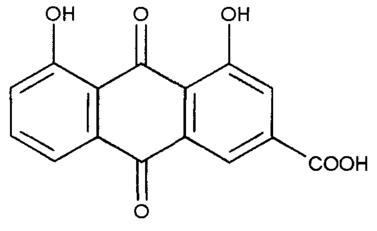


**Fig. (5).** Structure of rhein

Kuo *et al.*, [18] investigated the structure-activity relationships of anthraquinones on intestinal motility, using rabbit small intestinal strips. This study revealed the critical requirement of a hydroxy group at 2 position, whereas the presence of other polar groups at this position, such as an amino, aldehyde and carboxylic acid groups, significantly reduced the activity. The presence of a methyl group and esterification of the carboxylic acid at 2 position was found to abolish the activity.

## ANTINEOPLASTIC ACTIVITY

Folk medicine has attributed antitumor properties to preparations from medicinal plants containing quinones. Quinones for the anthraquinone, naphtoquinone and benzoquinone group were studied for their inhibitory growth effect on cultured malignant cells, which include cultured ovarian, breast, prostate, melanoma, lung, colon and pancreatic cancer cells.

For example, emodin, Fig. (4) is an active component from the root and rhizome of *Rheum palmatum* L. that has been reported to exhibit antitumor effects, but the mechanism is not known. Several studies demonstrated that emodin induces cell apoptosis in human lung squamous carcinoma cell line

CH27 and on cells derived from human colon carcinoma [19-22]. *Rheum officinale* H. Bn., a Chinese medicinal plant, also contained large amounts of anthraquinones as active compounds, such as emodin, Fig. **(4)**, chrysophanol, Fig. **(3)** and rhein, Fig. **(5)**, which specifically inhibited one of the carcinogenesis-related enzymes (cytochrome P450) [23]. Additionally, chrysophanol, emodin and rhein have been shown to inhibit benzo(α)pyrene-mediated DNA damage in human hepatoma cell line Hep62. In the same way, Kuo *et al.* [24] tested four anthaquinones purified from another Chinese herb *Polygonum hypoleucum* Onwi (emodin, Fig. **(4)**, emodin-1-glucoside, physcion, Fig. **(6)** and physcion-1-glucoside) for their effects on human mesangial cell proliferation *in vitro*. On a percentage basis, emodin had the highest suppressing activity on cells proliferation.

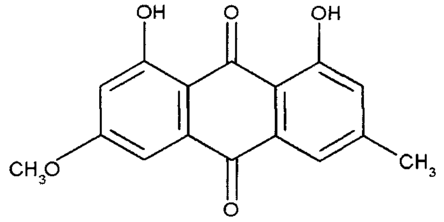


**Fig. (6).** Structure of physcion

Another natural anthraquinone, aloe-emodin, Fig. **(2)** present in *Aloe vera* L. leaves, has a specific *in vitro* and *in vivo* antineuroectodermal tumor activity. Aloe-emodin has been reported to be non-toxic for normal cells, but to possess specific toxicity for neuroectodermal tumor cells. Taking into account its unique cytotoxicity profile and mode of action, aloe-emodin might represent a conceptually new lead antitumor drug [25]. More recently, a free-floating cell line has been established from a metastatic lesion of a Merkel cell carcinoma patient. The cell line was characterized by immunocytochemical reactions with antibodies against the epithelial and neuroendocrine antigens. Aloe-emodin significantly inhibited the growth of Merkel carcinoma cells, meriting further investigation as a potential agent for treating these tumors [26].

Previously, Grimaudo *et al.* [27] had studied the effects of five purified anthraquinones from this plant on human K562 leukaemia, and only aloe-emodin, Fig. **(2)** produced reproducible antitumor effects. Research on the antitumor activity of compounds extracted from *Aloe vera* probably deserves to be continued. Shimpo *et al.* [28] examined the modifying effect of the whole leaf of another species of this genus, *Aloe arborescens* Miller var. *nathalensis* Berger on azoxymethane-induced aberrant crypt foci, putative pre-neoplastic lesions in the rat colorectum, suggesting that this plant has a chemopreventive effect against colon carcinogenesis, at least in the initiation stage.

Several studies demonstrated that aloin, another natural anthraquinone with potential antitumor activity, was effective on mice bearing solid Erlich carcinoma [29-31]. The antitumor activity of aloin was also examined against two epithelial-type tumor cell lines: breast and ovarian [32]. Other natural anthraquinones isolated from the roots of *Morinda elliptica* Ridl. were assayed for their cytotoxic activities. Of these, only damnacanthal was very cytotoxic against the breast carcinoma MCF-7 and T-lymphoblastic leukaemia CEM-SS cell lines [33].

The number of hydroxy groups in the anthraquinone nucleus seemed to play an important role in the degree of cell growth inhibition. Anthraquinones with two or three hydroxy groups were more effective than those with no hydroxy groups such as 9,10-dioxoanthracene [19]. Edenharder *et al.* [34] demonstrated that several anthraquinones and structurally related monoketonic compounds inhibited mutagenicity induced by 2-amino-3-methylimidazol[4,5-f]-quinoline in *Salmonella typhimurium* TA 98. A carbonyl function was a prerequisite for antimutagenicity, while, in general, anthraquinones were more potent antimutagens than structurally-related monoketonic compounds.

A series of anthraquinones has been chemically synthethized and the influences of structural features, such as the different functionalities in the nucleus have been studied in relation to cytotoxicity toward neoplastic cells. 1,3-dihydroxy-9,10-anthraquinone synthetic derivatives were tested *in vitro* for inhibition against several different human cancer cell lines. Structure-activity analysis indicates that epoxidation of the hydroxyanthraquinone increased cytotoxicity against tumor cells, but ring-opening of the epoxide

group with amine did not enhance cytotoxic activity [35]. In the same way, 2-(1-hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinone derivatives were chemically prepared and tested for their antitumor activity. Some of them showed remarkable activity [36]. Additionally, novel esters of chlorambucil with 2-(1-hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinone were synthesized and tested for their antitumor activity in mice bearing S-180 ascitic cells, as well as for cytotoxic activity against L1210 cells. Eight of them were highly cytotoxic [37].

The amino-substituted anthracene-9,10-dione derivatives also represent one of the most important classes of potential antitumor agents [38]. Recently, Zagotto *et al.* [39] have synthesized a new class of D- and L-aminoacyl-anthraquinone derivatives, and have tested these new compounds as cytotoxic agents. These studies have correlated their activity with the configuration of the chiral aminoacyl moiety [40,41]. Molecular modelling has been carried out for a number of amine-functionalised anthraquinone derivatives to determine the extent of their binding to DNA and their ability to inhibit the enzyme telomerase. The findings suggest that anthraquinone derivatives of this type inhibit telomerase [42,43].

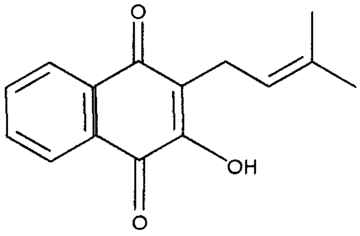


**Fig. (7).** Structure of lapachone

Naturally-occurring quinones of the naphthoquinone group also exert potent antineosplastic activity, and it is interesting highlight that the most studied compound is lapachone, Fig. (7). Pyranonaphthoquinones such as α- and β-lapachone have diverse biological activities and there has recently also been an increased interest in their anticancer activity. β-Lapachone, a plant

product obtained from plants of the *Tabebuia* genus, has been shown to be a novel inhibitor of DNA topoisomerase I, with a different mode of action from camptothecin and a chemical structure distinct from those of current anticancer drugs [44,45]. β-Lapachone has been found to induce apoptosis in various human cancer cells. Several studies demonstrated that β-lapachone induces cell death with the characteristics of apoptosis in human and rat prostate cancer cell lines [46,47]. This effect of β-lapachone was also observed in human promyelocytic leukaemia cell line HL-60 [48] and human multiple myeloma cell lines [49]. Weller *et al.* [50] also find that β-lapachone is much more potent than camphothecin in inducing acute cytotoxic effect on human malignant glioma cells. Among other human carcinoma and adenoma cell lines tested, human breast and ovary carcinoma [51], human hepatoma cell line HepA2 [52] and nasopharyngeal tumor cells KB [53] showed sensitivity to the cytotoxic effect of β-lapachone. These results suggest that β-lapachone is a potential compound to be added to cancer chemotherapy, particularly for prostate cancer. In addition, ablation of tumor colonies was seen in a wide spectrum of human carcinoma cells in culture after treatment with the combination of β-lapachone and taxol. This combination therapy has usually potent antitumor activity against human ovarian and prostate tumor in mice [54].

A series of β-lapachone and related naphthoquinones of natural and synthetic origin are being evaluated against drug-sensitive and drug-resistant cell lines and purified human DNA topoisomerases [55-57]. For example, a regio- and stereospecific synthesis of monoarylimino *o*-quinones derived from β-lapachone was achieved. Preliminary *in vivo* testing in assays against a standard panel of human tumor cell lines showed that several of these compounds had good scores with net cell kills [58].

The ability of other naturally-occurring naphthoquinone derivatives as antitumor agents is well documented. Naphthoquinones bearing at least one phenolic hydroxyl group, such as alkannin, Fig. **(8)** and shikonin, Fig **(9)**, are potent inhibitors of topoisomerase I [59,60]. Other investigations reported the ability of juglone, Fig. **(10)** and plumbagin, Fig. **(11)** to protect animals against chemically-induced neoplasia, such as a solid tumor (sarcoma-180) and Ehrlich ascites model [61,62].

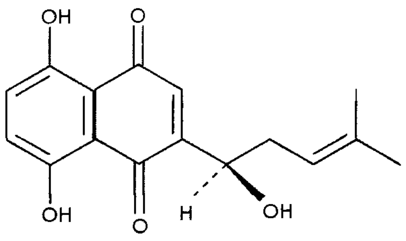


**Fig. (8).** Structure of alkannin

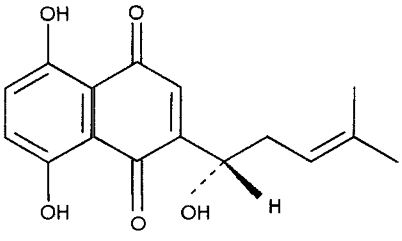


**Fig. (9).** Structure of shikonin

Other natural naphthoquinones, such as menadione, 1,4-naphthoquinone, 3-methyl-1,4-naphthoquinone and 2,3-dimethoxy-1,4-naphthoquinone presented antitumoral activity against the human colon carcinoma cell lines Caco-2 and HT-29 [63] and in human hepatoma cell lines [64]. More recently, Huang *et al.* [65] studied the effects of 2-mercaptophenyl-1,4-naphthoquinone, menadione and 1,4-naphthoquinone on cell proliferation and ionic currents in pituitary GH(3) lactotrophs. These studies suggest that the blockade of these ionic channels by these compounds may partly explain their inhibitory effect on the proliferation of GH(3) cells.

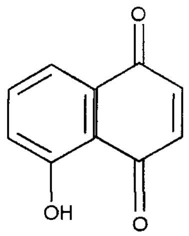


Fig. (10). Structure of juglone

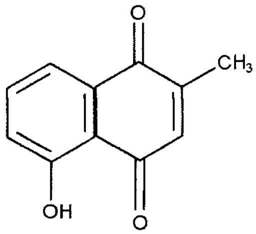


Fig. (11). Structure of plumbagin

In the search for new antitumor quinones, a series of naphthoquinone derivatives were synthesized and studies on their cytotoxicities and structure-activity relationships are being conducted. The inhibition of DNA topoisomerase I and the cytotoxic activity against L1210 cells of naphthoquinones showed the same order: 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) > 6-(1-hydroxyethyl)-DMNQ > 2-(1-hydroxyethyl)-DMNQ. The steric hindrance of the substituents must be the main reason the bioactivities are lower [66]. 6-(1-azidoalkyl)-DMNQ derivatives compared with 2-(1-azidoalkyl)-DMNQ isomers exhibited higher cytotoxic activity against L1210 mouse leukaemia cells and stronger inhibition of DNA topoisomerase I, confirming involvement of steric hindrance [67]. Several DMNQ derivatives which bear an unsaturated alkyl side chain with an ester bond were synthesized and tested for cytotoxic activity on L1210 cells. The introduction of the alkenoyl moities resulted in the enhancement of their cytotoxicities [68,69]. In the same way, a series of 6-(1-acyloxyalkyl)-DMNQ derivatives were synthesized and examined for their inhibitory effect on DNA topoisomerase I and their antiproliferative activity against L1210 cells. It was found that acylation of 6-(1-hydroxyalkyl)-DMNQ derivatives

possessing alkyl chains of C-2 and C-5 enhanced both bioactivities, suggesting that an increase of electrophilicity in the quinoid moiety makes the electrophilic acylation of bionucleophiles more favorable [70]. In addition, natural products of the naphthoquinone spiroketal structural type served as lead structures for the development of novel inhibitors of two breast cancer cell lines [71].

Gokhale *et al.* [72] have synthesized a series of hydroxynaphthoquinone metal complexes as antitumor agents. The cytotoxic studies against the human breast cancer cell line MCF-7 revealed enhanced activities for the metal complexes. The highest activity is observed for the copper compound of lawsone, Fig. **(12)** (2-hydroxy-1,4-naphthoquinone).

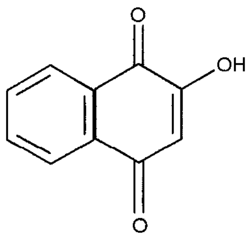


**Fig. (12).** Structure of lawsone

Additionally, several furanonaphthoquinones have shown useful activity in a yeat assay for DNA damaging agents and cytotoxicity in mammalian cell culture assays. These results, together with the planar aromatic character of the furanonaphthoquinone suggest that they may be acting as DNA intercalators [73,74]. In the same way, furanonaphthoquinones and analogues isolated from *Avicennia* plants having an alcoholic group in the dihydrofuran ring displayed the most potent antiproliferative activity and might be valuable as potent cancer chemopreventive agents [75]. In an attempt to improve this activity, various analogues containing a hydroxyamine chain have been synthesized [76].

Hypericin, Fig. **(13)** is a polycyclic quinone obtained from plants of *Hypericum* genus, which exhibits strong photodynamic antitumor effects.

Recent studies have demonstrated that this naphtodianthrone is a photosensitizing pigment that displays cytotoxic effects in neoplastic cell lines and that it is a powerful sensitizer for photodynamic therapy [77-81]. Photodynamic therapy has been described as a promising new modality for the treatment of cancer. Recently, Agostinis *et al.* [82] reviewed the current knowledge on the signalling pathways underlying the photocytotoxic action of hypericin. The mechanism of tumor eradication and mode of cell death induced by *in vivo* photodynamic therapy with hypericin involves vascular damage and apoptosis in the RIF-1 mouse tumor model [83].

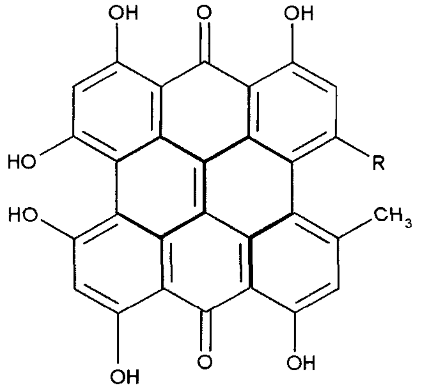


**Fig. (13).** Structure of hypericin

Finally, quinones from the benzoquinone group have also been reported to be antitumor agents against several tumoral cell lines, such as prostatic cancer [84] and human leukaemia K 562 cells [85].

## MUTAGENIC ACTIVITY

As we have seen in the last paragraph, various tests revealed that some quinones can have an antimutagenic effect, and thus that they could have some potential as anticancer agents. However, chronic abuse of anthranoid laxatives has recently been associated with an increased risk of colorectal

carcinoma. *In vitro* and animal studies have shown a potential role of anthranoid laxatives in both the initiation and promotion of tumorigenesis. Studies in humans have also suggested tumor-promoting activities for these laxatives [86,87]. This risk is particularly important in view of the wide abuse of self-administered laxatives for chronic constipation. There are data on the genotoxic potential of anthranoids and there is evidence of a tumorigenic potential in mice and rats. For example, anthraquinone glycosides of senna and cascara were investigated for their ability to induce aberrant crypt foci in the rat colon mucosa, which are considered to be putative preneoplastic lesions. These findings suggest that senna and cascara glycosides may behave as weak promoters in rat colon carcinogenesis [88]. Similarly, toxicological studies indicated that two hydroxyanthraquinones, aloe-emodin, Fig. **(2)** and emodin, Fig. **(4)**, present as minor components in senna, may represent a genotoxic or cancerogenetic risk for man [89].

Mueller *et al.* [90] investigated the genotoxicity of 1,8-dihydroxyanthraquinones present in Chinese herbs in the comet assay, the micronucleus test and the mutation assay in lymphoma L5178Y. Emodin, Fig. **(4)** was genotoxic whereas chrysophanol, Fig. **(3)** and physcion, Fig. **(6)** showed no effects. Previously, Mueller *et al.* [91] had reported that plant-derived 1,8-dihydroxyanthraquinone derivatives emodin and danthron were clearly genotoxic in mouse lymphoma L5178Y cells, whereas chrysophanol was only weakly genotoxic and physcion not at all. Furthermore, these studies had found that these compounds bound non-covalently to DNA and inhibited topoisomerase II activity. These data support the understanding that the genotoxicity of anthraquinones is, at least in part, mediated by non-covalent DNA-binding. Other investigations support the idea that inhibition of the catalytic activity of topoisomerase II contributes to anthraquinone-induced genotoxicity and mutagenicity [92,93]. There are studies to elucidate the enzymes involved in the biotransformation of naturally-occurring 1,8-dihydroxyanthraquinones and to investigate whether biotransformation may represent a bioactivation pathway. These data indicate that the cytochrome P450-dependent biotransformation of emodin and chrysophanol may represent bioactivation pathways for these compounds [94-96].

Additionally, 1-hydroxyanthraquinones present in certain medicinal plants such as *Rubia tinctorum* L. (madder root) are genotoxic agents and mice

colon carcinogens [97]. For example, lucidin, Fig. **(14)** (1,3-dihydroxy-2-hydroxymethyl-9,10-anthracenedione) and lucidin primeveroside were shown to be responsible for the potential genotoxic activity of madder root extracts. It suggested that the presence of two hydroxy groups in the 1,3 positions is a structural requirement for the genotoxicity of hydroxyanthraquinones such as lucidin [98,99]. Other investigations showed the ability of anthraquinones in promoting other neoplastic lesions such as skin tumor and cutaneous melanoma in mice [100,101] and on the bone marrow cells of mice [102]. Several benzo- and naphthoquinones have also been shown to be oxidative mutagens [103]. More recently, Guerra *et al.* [104] have investigated the teratogenic potential of lapachol, a naphthoquinone with therapeutic potential. Results have shown a strong abortifacient effect of lapachol in rats.

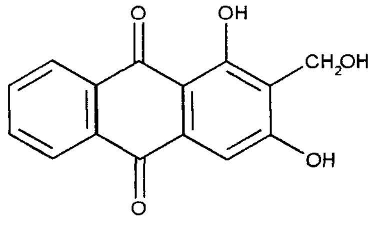


**Fig. (14).** Structure of lucidin

## ANTI-INFLAMMATORY ACTIVITY

Polyphenols, such as quinones, are major components of many traditional herbal remedies which exhibit several beneficial effects, including anti-inflammation. For example, Cuellar *et al.* [105] investigated the topical anti-inflammatory activity of extracts from *Cassia angustifolia* Vahl. and *Rheum palmatum*, medicinal plants commonly known for their anthraquinonic content, and used in traditional East Asian medicine against different skin disorders. All the extracts significantly inhibited the edema induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in both single or multiple applications.

Several studies were designed to elucidate anti-inflammatory action mechanisms of naturally-occurring anthraquinones. Wei *et al.* [106] investigated the *in vitro* anti-inflammatory activity of the isolated anthraquinone frangulin B, Fig. **(15)** from *Rhamnus formosana* Matsum, by determining their inhibitory effects on the chemical mediators released from mast cells, neutrophils, macrophages and microgial cells. This compound showed potent inhibitory effects on tumor necrosis factor-α (TNF-α) formation in lipopolysaccharide (LPS)/interferon-γ-stimulated murine microgial cell lines N9. In the same way, Kuo *et al.* [107] studied four anthraquinones isolated from *Polygonum hypoleucum* on primary human T lymphocytes. Emodin, Fig. **(4)** had the highest suppressing activity on cell proliferation, inflammatory cytokine production and calcium mobilization. These studies suggest that the inhibitory mechanisms of emodin on activated T cell proliferation are related to the impairment of cytokine production, interleukin-2 mRNA level and calcium in the cells.

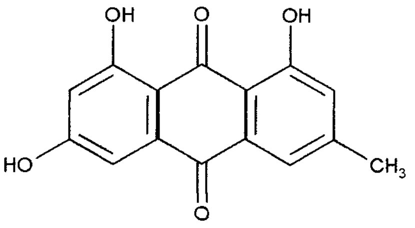


**Fig. (15).** Structure of frangulin

Interleukin-1β is a cytokine which plays a fundamental role in osteoarthritis pathophysiology and cartilage destruction. Targeting the activation mechanism on this cytokine appears to be important as a therapeutic approach. As the interleukin-1 converting enzyme is the physiological modulator of the production of active interleukin-1β, several studies have investigated the effect of two anthraquinones used in the treatment of osteoarthritic patients, diacerhein and its active metabolite rhein, Fig. **(5)**, on interleukin-1 expression and synthesis in human osteoarthritic cartilage. These results provide novel regulatory mechanisms by which

diacerhein and rhein could exert the effect on interleukin-1 in osteoarthritic cartilage [108,109]. Previously, Carney *et al.* [110,111] examined the effects of diacetylrhein and several related anthraquinone analogues on the synthesis, turnover and composition of cartilage in an *in vivo* experimental model of osteoarthritis. Diacetylrhein was well tolerated by the experimental animals, but did not produce significant changes in the synthesis or turnover of proteoglycans.

Pelletier *et al.* [112] evaluated the *in vitro* effects of diacerhein and its active metabolite rhein, Fig. **(5)**, on the production of nitric oxide (NO), prostaglandins (PGs), cyclooxygenase-2 (COX-2), as well as the production and expression of the inducible nitric oxide synthase (iNOS) in human osteoarthritic chondrocytes. Diacerhein and rhein are potent inhibitors of interleukin-1β induced NO production by chondrocytes and cartilage, without reducing $PGE_2$ production. Chen *et al.* [113] investigated the effect of other naturally-occurring anthraquinones on LPS-induced NO production and i-NOS and COX-2 gene expression in RAW2647 macrophages. The results indicate that only emodin concentration dependently inhibited LPS-induced NO production. On the other hand, Jun N-terminal kinase (JNK) is a stress-activated protein kinase that can be induced by inflammatory cytokines, bacterial endotoxins, osmotic shock, UV radiation and hypoxia. Several studies support targeting JNK as an important strategy in inflammatory disease. Bennett *et al.* [114] reported the identification of an anthrapyrazolone series with significant inhibition of JNK. These compounds inhibited the phosphorylation of JNK, the expression of inflammatory genes COX-2, interleukin-2, interpheron-γ, TNF-α, and prevented the activation and differentiation of primary human CD4 cell cultures.

Several observations suggest that naphthoquinones may also have effects on the production of inflammatory mediators, including cytokines. Fibroblasts are recognized as a rich source of cytokines, and Reddi *et al.* [115] have examined the effect of various natural naphthoquinones on the production of interleukin-6 by LPS-stimulated human gingival fibroblasts. Naphthoquinone vitamins, e.g. vitamin K, Fig. **(16)**, are widely recognized for their role in the γ-carboxylation of specific glutamyl residues in coagulation, anti-coagulation and extra-hepatic proteins. However, there have been reports that these compounds can exert actions other than those

normally associated with protein γ-carboxylation. These compounds are capable of inhibiting interleukin-6 production probably by a mechanism related to the redox capacity of these naphthoquinones. Other naturally-occurring quinones also have effects on the production of other inflammatory mediators such as protein kinase C [116,117], leukotriene $B_4$ (lipoxygenase activity) [118-120] and on phospholipase $A_2$ activity [121].

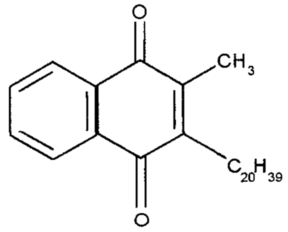


**Fig. (16).** Structure of vitamin K

A series of 2-substituted 3-chloro-1,4-naphthoquinones were synthesized and their anti-inflammatory activities were evaluated. The structure-activity relationships in this series were also examined. Most of the 2-alkyl/arylcarboxamido derivatives of 3-chloro-1,4-naphthoquinones showed potent activity [122,123]. Selected naphthoquinone compounds were investigated for 5-lipoxygenase inhibiting and antioxidative properties. There is a clear-cut correlation of both qualities in those compounds with a 3-hydroxy function and with two, one or without any tert-butyl group at the phenyl moiety [124].

## ANTIOXIDANT ACTIVITY

The antioxidant properties of naturally occurring anthraquinones and anthrones were evaluated using different model systems. For example, the antioxidant activity of these compounds was studied on the inhibition of peroxidation of linoleic acid. These results suggest that the antioxidant mechanism for two anthraquinones, emodin, Fig. **(4)** and aloe-emodin, Fig. **(2)**, possibly depends on scavenging hydroxy radicals, while the pro-oxidant

activity exhibited by chrysophanol, Fig. **(3)** might be due to the enhanced production of free radicals [125,126]. Several investigations reported the antioxidant actions of anthraquinone compounds found in *Rheum* and *Cassia* by measuring inhibition of pyrogallol autoxidation and radical scavenger activity against hydroxy radicals generated via Fenton reactions. Emodin was shown to be a potent inhibitor of superoxide radicals in contrast to other compounds [127,128].

More recently, Choi *et al.* [129] investigated the antioxidant activities of alaternin, Fig. **(17)** (2-hydroxyemodin) and emodin, Fig. **(4)** for their respective potential to inhibit lipid peroxidation in the linoleic acid system, to inhibit total reactive oxygen species generation in kidney homogenates, to inhibit peroxynitrite formation and to scavenge authentic peroxynitritres. These results indicate that alaternin is a potentially effective and versatile antioxidant and can be used to protect biological systems and functions against various oxidative stresses. Sheu and Chiang [130] investigated eighteen natural anthraquinones and related compounds for their inhibitory effects on xanthine oxidase. This enzyme catalyses the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. The results showed that anthrarobin and purpurin had moderate effects on xanthine oxidase inhibition, and both of them induced mixed type (competitive, non-competitive) inhibition with respect to the substrate xanthine.

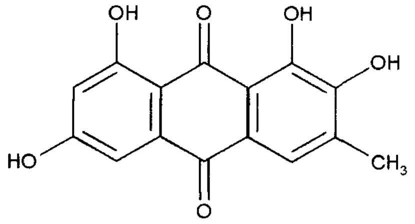


**Fig. (17).** Structure of alaternin

Antioxidant activity of other natural quinones, such as ubiquinone, phylloquinone and menadione was studied in the model system of the methyl oleate-initiated oxidation. The menadione antioxidant activity was shown to

be two-fold higher as compared with the inhibitory activity of other natural quinones [131]. In the reduced form, ubiquinone functions as a lipid-soluble antioxidant and protects cells from lipid peroxidation [132].

Kumbhar *et al.* [133] also investigated the modulation of radiation-induced lipid peroxidation in synaptosomes by iron (II) and iron (III) complexes of two naturally occurring and therapeutically relevant naphthoquinones: juglone, Fig. **(10)** and lawsone, Fig. **(12)**. At lower concentrations the complexes enhance lipid peroxidation, predominantly through redox cycling as observed for iron (II)-juglonate, while at higher concentrations the complexes tend to limit lipid peroxidation through fast recombination. The antioxidant properties of 2,3,5,7,8-pentahydroxy-1,4-naphthoquinone (echinochrome A) were linked with the scavenging of peroxy radicals in liposomes, trapping of superoxide anion radicals, and binding of ferrous ions to inactive complexes [134]. Other natural naphthoquinones, such as shikonin, Fig. **(9)**, show highly efficient antioxidative activities against several types of reactive oxygen species: singlet oxygen, superoxide anion radical, hydroxy radical and tert-butyl peroxyl radical, as well as iron-dependent microsomal lipid peroxidation. Based of the scavenging abilities of shikonin, the compound was concluded to react directly with reactive oxygen species and exhibits antioxidative activity which in turn exerts anti-inflammatory activity [135].

Several studies were also designed to evaluate the antioxidant properties of St. John's Wort (*Hypericum perforatum* L.), an herbal drug used in the treatment of burns, bruises, swelling and anxiety. Commercially available formulations of St. John's Wort, standardized to the naphthoquinone hypericin, Fig. **(13)**, inhibit free radical production in both cell-free and human vascular tissue [136].

Several quinonic compounds have been synthesized and their antioxidant activities are being evaluated. For example, a series of arylthiolated-2,3-dimethoxy-1,4-benzoquinones were chemically prepared and tested for their effect on the respiratory system and the lipid peroxidation in bovine heart mitochondria. These quinones were as efficient as exogenous ubiquinone for the inhibition of lipid peroxidation. 5- and 5,6-diarylthio groups on the quinone ring were found to be favorable for the inhibition of the respiratory system and lipid peroxidation [137]. Giorgini *et al.* [138] studied the

reactivity of several quinones and of the corresponding quinols toward carbon- and oxygen-centered radicals. All quinones bearing at least one nuclear position free readily react with alkyl and phenyl radicals to afford the alkylated quinones.

## ANTIPSORIATIC ACTIVITY

Some naturally occurring quinonic compounds appear to be promising as effective antipsoriatic agents, due to their potent activity against the growth of human keratinocytes. Antipsoriatic anthrones are among the most commonly used topical agents for the treatment of psoriasis. These drugs generate reactive oxygen species during their auto-oxidation under physiological conditions. Several studies have indicated that activation of molecular oxygen by anthrones may play a critical role in their mechanism of action at the molecular level. This mechanism is related to their redox activity leading to the production of active oxygen species which include singlet oxygen, superoxide anion radical and hydroxy radical [139,140]. In 1996, Muller [141] summarized the evidence pointing toward the significance of oxygen activation and radical formation in antipsoriatic action and induction of skin inflammation of anthrones.

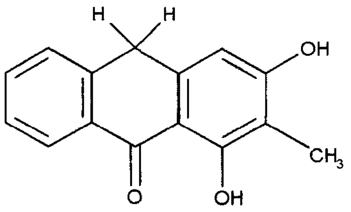


**Fig. (18).** Structure of anthralin

Anthralin, Fig. **(18)** is the most common therapeutic agent among a small number of pro-oxidant 9-anthrones, effective in the topical treatment of psoriasis. However, the usefulness of this drug is diminished by toxic side effects, including skin irritation and inflammation. Several studies suggest

that oxidative stress generated at the site of anthralin treatment alters the expression of dermal chemokines and other cytokines, resulting in the recruitment of inflammatory cells. Systemic antioxidant administration may provide opportunities for therapeutic intervention against anthralin-associated toxicities [142].

Heterocyclic substituted derivatives of the antipsoriatic anthralin were synthesized and evaluated *in vitro* for their antiproliferative action against keratinocytes and their ability to induce keratinocyte differentiation. The indole-2-carboxylic acid analogues exhibited the same excellent antiproliferative activity as anthralin and also induced terminal differentiation of keratinocytes. As a benefit of their strongly diminished potential to generate oxygen radicals, these synthetic compounds did not induce damage of keratinocyte membranes [143]. 10-arylthio derivatives of anthralin are also of interest in the search for new antipsoriatic agents. These synthetic compounds have been evaluated for their ability to inhibit the growth of the human keratinocyte cell line HaCaT. Biological tests have shown that 10-benzylthio-1,8-dihydroxy-9(10H)-anthracenone slightly inhibited the HaCaT cells [144,145]. Muller *et al.* [146] described the synthesis and structure-activity relationships of a series of novel 10-arylacetyl-1,8-dihydroxy-9(10H)-anthracenones. Several compounds were identified which are equally potent as inhibitors of human keratinocyte growth as the antipsoriatic agent anthralin. Additionally, Muller *et al.* [119] synthesized 10-phenylbutyryl-substituted anthracenones as inibitors of keratinocyte growth.

In the naphthoquinone group, a number of lapacho compounds, the most common naphthoquinonic constituents of the inner bark of *Tabebuia impetiginosa* (Mart. ex D. C.) Standl were evaluated *in vitro* against the growth of the human keratinocyte cell line HaCaT. All compounds inhibited keratinocyte growth and appear to be promising as effective antipsoriatic agent [147].

## ANTIVIRAL ACTIVITY

The development of new antiviral drugs, specially herbal preparations, remains desirable. Several studies screened natural quinones and plant extracts containing these compounds for inhibitory activity against several

DNA and RNA viruses. For example, 152 methanol and water extracts of different parts of 71 plants commonly used in Sudanese traditional medicine were screened for their inhibitory effects on hepatitis C virus protease using *in vitro* assay methods. Thirty-four extracts showed significant inhibitory activity. From the *Embelia schimperi* D. C. extract, two benzoquinones, embelin and 5-O-methylembelin, were isolated and found to be potent inhibitors [148,149].

Hypericin, Fig. **(13)**, a natural naphthoquinone of the common St. John's Wort plant *Hypericum perforatum* has *in vitro* activity against several viruses, including bovine diarrhoea virus, a pestivirus with structural similarities to the hepatitis virus [150]. Several investigations suggest that some plants of the genus *Hypericum* from Southern Brazil, such as *Hypericum connatum* Lam, *Hypericum caprifolium* Boiss and *Hypericum polyanthemum* Klotzsch ex Reichardt also contain compounds with potential antiviral activity against lentivirus [151].

Quinones with one, two and three aromatic rings are a new class of inhibitors of human immunodeficiency virus-1 (HIV-1) proteinase, an enzyme essential for replication of HIV and an important drug target in the treatment of acquired immunodeficiency syndrome (AIDS). For example, the trimeric quinone framework of conocurvone is crucial for the potent anti-HIV activity of this compound [152,153]. Recently, Emadi *et al.* [154] described a new synthesis of these trimeric quinones. Substituted anthraquinones bearing hydroxyl substituents as one of their three rings were the most potent of these inhibitors. In an effort to develop new drugs preventing the growth of HIV, Min *et al.* [155] developed an *in vitro* assay method of ribonuclease H (RNase H) activity associated with reverse transcriptase (RT) from HIV-1. Some naphthoquinones, such as 1,4-naphthoquinone, vitamin K, Fig. **(16)**, juglone, Fig. **(10)** and plumbagin, Fig. **(11)** moderately inhibited RNase H activity and others, including naphthazarin, Fig. **(19)** and shikonin, Fig. **(9)** showed weak inhibition. Diterpenoid quinones such as tanshinones and aryl-substituted naphtho- and anthraquinones also had moderate inibition against RNase H activity [156].

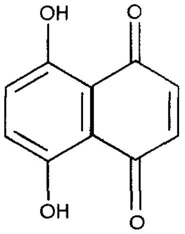


**Fig. (19).** Structure of naphtharazin

In a screening study with plant extracts, a rhubarb root extract with 4-6% hydroxyanthracene derivatives showed a promising activity for topical treatment of herpes labialis [157]. 31 herbs used in Chinese medicine were also screened to develop anti-herpes simplex virus type I (HSV-1) compounds from plants. These results showed that ethanolic extract of *Rheum officinale* prevented the process of virus attachment and penetration, and has a potential value as source of new powerful anti-HSV compounds [158]. The proteases encoded by the herpes virus, including HSV-1 and human cytomegalovirus (HCMV) are attractive targets for antiviral drug development because of their important roles in viral replication. 1,4-dihydronaphthalene and naturally-occurring naphthoquinones and anthraquinones were found to be potent inhibitors of HSV-1 protease [159-161]. Additionally, a random screening approach has identified 2-chloro-3-substituted-1,4-naphthoquinones as potent inactivators of HCMV protease [162].

Natural quinones also exert antiviral activity against other viruses, such as the Epstein-Barr virus and poliovirus type 2 and 3 [163-165]. For example, chrysophanic acid (1,8-dihydroxy-3-methylanthraquinone) isolated from the Australian Aboriginal medicinal plant *Dianella longifolia* Street has been found to inhibit the replication of poliovirus type 2 and 3 *in vitro*. Four structurally-related anthraquinones (rhein, Fig. **(5)**, 1,8-dihydroxyanthraquinone, emodin, Fig. **(4)** and aloe-emodin, Fig. **(2)**) were also tested for activity against poliovirus type 3, although none of them was as active as chrysophanic acid against the virus [166].

## ANTIFUNGAL ACTIVITY

In natural products, this activity is usually associated with the presence of phenolic functions. Investigations have been conducted to study the mechanism of action of antifungal quinones, particularly naphthoquinones, towards several fungi such as *Candida albicans* [167,168] and *Fusarium* spp. [169]. The growth of the white-rot basidiomycete *Pleurotus sajor-caju* in malt-agar plates was also inhibited by three naturally-occurring plant-derived naphthoquinones: juglone, Fig. **(10)**, lawsone, Fig. **(12)** and plumbagin, Fig. **(11)** [170].

Mahoney *et al.* [171] investigated the effect of a series of naphthoquinones (1,4-naphthoquinone, juglone, Fig. **(10)**, 2-methyl-1,4-naphthoquinone and plumbagin, Fig. **(11)**) on fungal viability and aflatoxigenesis. Aflatoxins are metabolites produced on infection with *Aspergillus flavus*. The naphthoquinones delayed germination of the fungus and were capable of completely inhibiting growth at higher concentrations. Structural features associated with decreased fungal viability and the greatest effect on aflatoxigenesis are the presence of a 5-hydroxyl or 2-methyl substituent, but there is no significant additive effect when both of these substituents are present. Lee *et al.* [172] investigated the effects of naturally-occurring compounds from plants on biotransformation of aflatoxin B-1. These results demontrate that some anthraquinones may have significant inhibitory effects on the metabolic transformation of aflatoxins to their hepatotoxic or carcinogenic derivatives or, alternatively, may promote their transformation into nontoxic products.

In the anthraquinone group, rhein, Fig. **(5)**, physcion, Fig. **(6)**, aloe-emodin, Fig. **(2)** and chrysophanol, Fig. **(3)** isolated from *Rheum emodi* L. rhizomes exhibited antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* [173]. Different extracts containing anthraquinones of both fresh and dry leaves of *Aloe eru* Berger, *Aloe vera* and *Aloe arborescens* were also screened for their antifungal activity against *Aspergillus niger*, *Cladosporium herbarum* and *Fusarium moniliforme* [174].

## ANTIBACTERIAL ACTIVITY

Several investigations have reported the study of the activity of quinone derivatives and plant extracts containing these compounds against several pathogenic bacteria. *In vitro* antibacterial testing was carried out using the disk diffusion method against Gram-positive *Staphylococcus aureus*, *Staphylococcus epidermis* and Gram-negative *Pseudomonas aeruginosae*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Escherichia coli*. Ethanol extracts of *Cassia occidentalis* L. and *Cassia alata* L. and metabolite-rich fractions (anthraquinones and sennosides, Fig. (**1**)) of leaves, pods, flowers and callus have been tested against indicator human pathogenic bacteria. The anthraquinones were found to be more active against *Escherichia coli* and *Staphylococcus aureus*, while sennosides were most active against *Aspergillus flavus* [175,176].

Hatano *et al.* [177] also studied the effect of anthraquinone derivatives isolated from *Cassia tora* L. on *Escherichia coli* K12, *Pseudomonas aeruginosae* and some strains of *Staphylococcus aureus*. Among them, torachrysone, toralactone, aloe-emodin, Fig. (**2**), rhein, Fig. (**5**) and emodin, Fig. (**4**) showed noticeable antibacterial effects. Some quinoid compounds such as aloe-emodin and thymoquinone showed potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Aloe-emodin was basteriostatic while thymoquinone was bactericidal. The structure-function relationship study suggests that hydrophilic groups at position 3 of anthraquinone and methyl groups of benzoquinones are very important for their activity [178]. More recently, Manojlovic *et al.* [179] investigated the antimicrobial activity of an extract and isolated anthraquinones from *Caloplaca schaereri* (Flöcke) Zahlbr.

In the naphthoquinone group, two dimeric naphthoquinones, diospyrin and isodiospyrin isolated from the root of *Diospyros piscatonia* (Gurke) and *Euclea natalensis* D. C., common ingredients in several folk medicines, have been shown to have a broad spectrum of antibacterial activity, e.g., against *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella choleraesuis* serotype *typhi*, *Mycobacterium chelonae* and *Mycobacterium tuberculosis* [180,181]. Yang *et al.* [182] isolated a single bioactive compound, 2-methoxy-1,4-naphthoquinone, from the ethanol extract of the dried aerial

parts of *Impatients balsamita* L. Its antomicrobial activity was evaluated using 12 bacterial strains. Five Gram-positive and two Gram-negative bacteria were highly sensitive to this compound. Mehrabian *et al.* [183] detected the antimicrobial effects of an extract containing naphthoquinonic compounds from *Rubia tinctorum* and *Juglans regia* L. on some bacteria such as *Bacillus subtilis*, *Bacillus cereus* and *Bacillus mycoides*. The antimicrobial effects are microbicidal. Ethanolic extracts of 20 selected plants species used by Yemeni traditional healers to treat infectious diseases were also screened for their antibacterial activity against both Gram-positive and Gram-negative bacteria. The ethyl acetate extract of *Lawsonia inermis* L., known to contain naphthoquinonic compounds, was found to be the most active against all bacteria in the test system [184].

Rothery *et al.* [185] have used two hydroxylated naphthoquinol analogues, reduced plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) and reduced lapachol (2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone) as substrates from *Escherichia coli* anaerobic reductases. Additionally, the 2-hydroxy-N-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine revealed good activity against *Staphylococcus aureus* [186]. Relationships between the chemical structure and antibacterial activity of these compounds was studied. Introduction of a second methyl group in C-3 of the isoxazol ring reduced the antibacterial activity, while a second aminoisoxazolyl function in C-2 of the naphthoquinone ring inhibited the biological activity [187]. Several 1,4-naphthoquinone derivatives having a hydrazino side chain were synthesized from diazo-naphthalene-1,2,4-trione and tested as potential antimicrobial agents against *Staphylococcus aureus* [188].

In the benzoquinone group, inhibition assays using *Escherichia coli* and *Saccharomyces cerevisiae* revealed the inhibitory effects of 1,4-benzoquinone and toluquinone against all tested microorganisms [189].

## PROTOZOOCIDAL ACTIVITY

The protozoocidal activity of quinone derivatives was evaluated *in vitro* against *Plasmodium*, *Leishmania*, *Trypanosoma*, *Trichomonas* and *Toxoplasma*. Malaria is a major tropical diseases which kills two million people annually. The population at risk from this disease has increased

because of the difficulties in erradicating the mosquito vector in the endemic regions, and the emergence and spread of parasite resistance to all the commonly used antimalarials.

Lapachol is a naphthoquinone from *Tabebuia* spp. used as an antimalarial agent with strong activity against *Plasmodium falciparum* [190]. Four naphthoquinones from *Kigelia pinnata* D. C. rootbark were assessed *in vitro* against chloroquine-sensitive (T9-96) and-resistant (K1) *Plasmodium falciparum* strains. Isopinnatal, kigelinol and isokigelinol exhibited lower activity against both strains [191]. More recently, Kapadia *et al.* [192] have identified aminonaphthoquinones as a class of antimalarial compounds with activity against *Plasmodium falciparum*. Among these compounds, 2-amino-3-chloro-1,4-naphthoquinone is the most potent. Another medicinal plant such as the root of *Nephentes thorelii* L. yielded plumbagin, Fig. **(11)**, 2-methylnaphthazarin and droserone, Fig. **(20)**, with antimalarial potential [193]. Other antimalarial naphthoquinones such as atovaquone inhibit malarial dehydroorotate dehydrogenase by inhibiting electron transport [194]. Atovaquone is a chemotherapeutic agent used to treat pneumonia caused by *Pneumocystis carinii* in some immunocompromised patients [195].

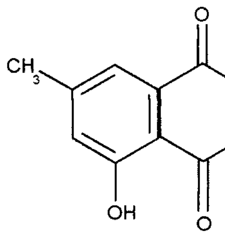


**Fig. (20).** Structure of droserone

A series of monomeric and dimeric naphthoquinones with potential for treatment of *Leishmania* infections were identified *in vitro* using a direct cytotoxicity assay against extracellular promastigotes of *Leishmania* spp. [196,197]. Lapachol was evaluated *in vitro* against intracellular amastigotes of *Leishmania braziliensis* and then tested in an animal model (hamster) to try to reproduce the leishmanicidal activity. *In vitro* lapachol exhibited an

anti-amastigote effect, whereas *in vivo* it did not prevent the development of *Leishmania*-induced lesions [198].

Cryptotanshinone is a quinoid diterpene with a nor-abietane skeleton isolated from roots of the Iranian medicinal plant *Perouskia abronatoides* L. which exhibited leishmanicidal activity *in vitro*. These findings provide a rationale for the traditional use of the roots in Iran as a constituent of poultices for treatment of cutaneous leishmaniasis [199]. More recently, Valderrama *et al.* [200] synthesized several eurylfurylquinones and hydroquinones from activated monosubstituted 1,4-benzoquinones and studied their *in vitro* activities against *Leishmania amazonensis*.

Additionally, several naphthoquinones showed remarkable anti-trypanosomal activity, such as four naphthoquinones isolated from *Kigelia pinnata*: 2-(1-hydroxyethyl)-naphthol[2,3-b]-furan-4,9-quinone, isopinnatal, kigelinol and isokigelinol. These compounds were assessed for anti-trypanosomal activity against *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* bloodstream form trypomastigotes *in vitro* [201]. These types of naphthoquinones, isolated from *Calceolaria sessiles* D. C. were tested against *Trypanosoma cruzi* epimastigotes and they produced a temporary increase of oxygen consumption in *Trypanosoma cruzi*, suggesting the generation and participation of free radicals [202]. With the aim of understanding the influence of redox potentials on the trypanocidal activity, a series of quinones were tested *in vitro* with trypomastigotes of *Trypanosoma cruzi* [203]. Trypanothione reductase is both a valid and attractive target for the design of new trypanocidal drugs. Starting from menadione, plumbagin, Fig. **(11)** and juglone, Fig. **(10)**, three distinct series of 1,4-naphthoquinones were synthesized as potential inhibitors of trypanothione reductase from *Trypanosoma cruzi* [204-206]. Naphthol[2,3-b]-thiophen-4,9-quinone and derivatives, and pyranobenzoquinones were also chemically prepared and evaluated for their trypanocidal activity [207,208].

Naphthoquinones isolated from the wood of trees of the families Bignoniaceae and Verbenaceae have been subjected to an interdisciplinary study since the seventies, when Dr. Gilbert, at the Federal University of Rio de Janeiro, launched a program on the chemistry of natural products active against endemic diseases. Recently, De Moura *et al.* [209] have described the synthesis of five naphthoimidazoles derived from this program and their

activity towards *Trypanosoma cruzi*. The biological activities of the naphthoquinone lapachol and its cyclization product β-lapachone extracted from trees of the genus *Tabebuia*, have also been intensively studied. In studies on heterocyclic derivatives obtained from the reaction of these naphthoquinones with amino-containing reagents, 22 derivatives of β-lapachone, nor-β-lapachone and lapachol were synthesized and their activities against trypomastigote forms of *Trypanosoma cruzi* were evaluated [210]. Previously, Pinto *et al.* [211] reported the biological activity of allyl derivatives of lawsone, Fig. **(12)**, a natural naphthoquinone from *Lawsonia alba* Lam. inactive against *Trypanosoma cruzi*. The introduction of an allyl group in lawsone gives rise to O-allyl-lawsone and C-allyl-lawsone that have shown activity against the parasite. In the anthraquinone group, aloe-emodin, Fig. **(2)** was the only compound active against *Trypanosoma brucei* [212].

Finally, two 3-alkyl-substituted-2-hydroxy-1,4-naphthoquinones were evaluated for activity against *Toxoplasma gondii in vitro* and in murine models of acute toxoplasmosis. These results indicate that treatment with these compounds have promising activity [213].

## MOLLUSCICIDAL ACTIVITY

Several studies have investigated the potential of natural naphthoquinones as the basis for a new class of pest control agents. For example, structure-activity studies on two pesticidal naphthoquinones from *Calceolaria andina* Benth. both containing a key structural feature not previously investigated, led to the identification of analogues with commercial levels of activity [214,215]. A series of compounds with structures based on these naphthoquinones have been synthesized. A feature of the series is the lack of resistance shown by strains resistant to established classes of pesticides. The tetra-substituted carbon atom in the side-chain is important for this activity as can be observed in natural products. Activity of the compounds examined was particularly high against *Bemisia tabaci* and *Tetranynchus urticae* in direct-contact tests, but was much lower than expected in leaf-dip test [216].

Dos Santos *et al.* [217,218] tested the activity of lapachol and other 2-hydroxy-3-alkylnaphthoquinones possessing nitrogenated alkyl chains against the snail *Biomphalaria glabrata*. Lapachol, isolapachol and nor-

lapachol showed strong molluscicidal activity against the adult snail. As the derivatives can be synthesized without any difficulty, large-scale synthesis and field tests can be conducted, with a view to large-scale molluscicidal programs. The main mode of herbicidal activity of 2-hydroxy-3-alkyl-1,4-naphthoquinones has been shown to be inhibition of photosystem II. Other modes of action were also investigated using two representative compounds of this group. They did not show any activity on photosystem I or mitochondrial complex I, or generate toxic oxygen radicals by redox cycling reactions [219].

Weissenberg *et al.* [220] investigated the effect of substituted and ring changes in naturally occurring naphthoquinones on the feeding response of the larvae of the Mexican bean beetle *Epilachna varivestis*. Among the model compounds, 2-chloro-3-amino-1,4-naphthoquinone and α-naphthylamine displayed appreciable activity. More recently, Magdum *et al.* [221] investigated the molluscicidal activity of two naturally occurring naphthoquinones, plumbagin, Fig. **(11)** from *Plumbago* spp. and juglone, Fig. **(10)** from *Juglans regia*, using the red cotton bug *Dysdercus koenigii*. Their activity was compared with synthetic naphthoquinones, two benzoquinones (2,6-dimethyl- and 2,3,6-trimethylbenzoquinone) and a hydroquinone (2,6-dimethylhydroquinone). Among the natural products, juglone induced more sterilizing effects than plumbagin. Plumbagin and azadirachtin also alter the activity profiles of two lysosomal enzymes in the fat body of *Helicoverpa armigera* [222]. Additionally, anthraquinones from *Rheum palmatum* and *Rumex dentatus* L. have shown molluscicidal activity against the schistosome vector snails *Oncomelania*, *Biomphalaria* and *Bulinus* [223].

## ANTIDEPRESSANT ACTIVITY

Tricyclic antidepressants are the mainstay of treatment of painful polyneuropathy, but cannot be used in a substantial number of patients. St. John's Wort (*Hypericum perforatum*) is a herbal antidepressant which may act via mechanisms similar to tricyclics. Clinical trials have extensively reported the ability of *Hypericum perforatum* extracts to exert a significant antidepressant activity [224-226]. Hypericins, Fig. **(13)** are considered to be one of the compounds contributing to the activity of the extract [227]. These

naphthodianthrones exist in various forms in the *Hyperici* herb. Protopseudohypericin and protohypericin (protopigments) are converted into pseudohypericin and hypericin (pigments) under the action of light [228-230].

Several investigations have studied the *in vivo* antidepressant activity of this herb and of compounds isolated from it. For example, a commercially available extract of the aerial parts of *Hypericum perforatum* L1160 and hypericin, Fig. **(13)** showed pronounced activity in selected animal bioassays. These include the forced swimming test and the tail suspension test, used to determine antidepressant activity, and tests indicating activity on the central nervous system, such as body temperature and ketamine-induced sleeping time [231,232].

*Hypericum perforatum* is one of the leading psychotherapeutic phytomedicines, and because of this great effort has been devoted to clarifying its *in vitro* mechanism of action. *Hypericum* is a nonspecific inhibitor of the neuronal uptake of monoamines: serotonin (5-HT), noradrenaline (NA), dopamine (DA) as well as ν-aminobutyric acid (GABA) and glutamate [233,234]. Cott [235] and Raffa [236] investigated a crude *Hypericum* extract and a sample of pure hypericin in a battery of *in vitro* receptor assays, and two enzyme assays. Hypericin had affinity only for NMDA receptors while the crude extract had significant receptor affinity for adenosine (nonspecific), GABA A and B, benzodiazepine, inositol triphosphate and monoaminooxidase A and B. Several studies have investigated the possible involvement of receptors and of serotonergic mechanisms in the effects of *Hypericum perforatum* extract containing 0.3% hypericin, on immobility time in the forced swimming test. These results suggest that the antidepressant-like effect of *Hyperici* herb in the forced swimming test may be mediated by interaction with receptors and to some extent by increased serotonergic neurotransmission [237-240]. Simmen *et al.* [241] studied the effect of extracts and some constituents of St. John's Wort at various central nervous system receptors. Binding inhibition was examined for the G-protein coupled opiod, 5-HT, histamine, neurokinin and corticotropin releasing factor receptors, for the steroid strogen-receptor and for the ligand-gated ionchannel GABA A receptor. Hypericin showed the most potent binding inhibition of all tested constituents.

More recently, Butterweck *et al.* [242] have investigated the chronic effects of St. John's Wort and hypericin on regional brain amine metabolism. These data clearly show that long-term, but not short-term, administration of St. John's Wort and its active constituent hypericin modify levels of neurotransmitters in brain regions involved in the pathophysiology of depression. Previously, Butterweck *et al.* [243] used in situ hybridization histochemistry to examine in rats the effects of short-term (two weeks) and long-term (eight weeks) administration of *Hypericum* extract and hypericin on the expression of genes that may be involved in the regulation of the hypothalamic-pituitary-adrenal axis. Select stress-induced changes in gene transcription in particular brain areas can be prevented by long-term treatment with the herbiceutical St. John's Wort.

Recent pharmacologic evidence suggests that other constituents of this plant, apart from hypericins, may be of greater importance in the reported psychotherapeutic activity. Phenylpropanes, flavonol derivatives, biflavones, proanthocyanidins, xanthones, phloroglucinols, some aminoacids, naphthodianthrones and essential oil constituents are the natural plant products known from the crude drug of *Hypericum perforatum*. These compounds are discussed with respect to their possible contribution to the clinically-demonstrated antidepressant efficacy of extracts obtained from the *Hyperici* herb [244-254].

Despite the wide use of St. John's Wort as an antidepressant, recent investigations have reported a probable drug interaction between this herb and/or hypericin and several drugs [255-258]. Recent clinical studies have demonstrated that *Hypericum* extracts increase the metabolism of various drugs, including combined oral contraceptives, cyclosporin, indinavir and digoxin. St. John's Wort was reported to substantially decrease blood/plasma concentrations and the efficacy of these drugs. These results indicate a direct inducing effect of St.John's Wort on intestinal P-glycoprotein/MDR1 (in rats and humans), hepatic cytochrome P450 3A2 (in rats), and intestinal and hepatic cytochrome P450 3A4 (in humans) [259-261]. Several studies have been conducted into the potential of its interaction with other drugs due to the induction of cytochrome P450 isoenzymes 1A2 and and 3A4 by the component hypericin. *In vitro* assays indicate that hypericin is a potent antagonist of cleavage complex stabilization by the chemotherapeutics

etoposide and amsacrine. This antagonism appears to be due to the ability of hypericin to intercalate or distort DNA structure, thereby precluding topo II binding and/or DNA cleavage [262].

## OTHER ACTIVITIES AND USES

As we can see in the present review, plant quinones are of interest in many respects. Preparations and products derived from natural quinones, and their synthetic relatives, serve medicinal, therapeutical and technological purposes. In technology, the range of interest extends from timber and dye material to semiconductors. Among the possible new materials for microelectronics, quinones have a number of significant advantages. Similarly, polymers with quinone functionality possess biodegradability and are promising candidates for functional materials in the future. In the search for the polymers of natural products, a natural quinone extracted from *Embelier libes* D. C., embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone), were electrochemically polymerized and their properties investigated [263,264].

The usefulness of the intensively-coloured naturally-occurring quinones as dyes (e.g., madder, kermes or cochineal) was recognised early in civilisation. Common madder (*Rubia tinctorum*) produces anthraquinone pigments in its roots, one of them being alizarin, Fig. **(21)** (1,2-dihydroxy-anthraquinone) which has been used for dyeing textiles since ancient times, and has an important industrial value [265]. The leaves of *Lawsonia inermis* (popularly named as henna) have also been used since ancient times for decorating and dyeing hands, soles, beard and hair and to impart beautiful shades of a dark red color. The naphthoquinone lawsone, Fig. **(12)**, a brown powder, was isolated from the leaves of henna and was used as a staining agent [266].

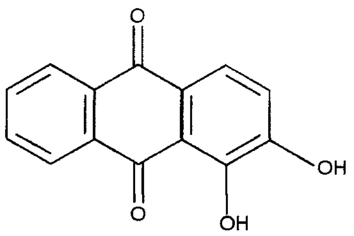


**Fig. (21).** Structure of alizarin

Carmine, Fig. **(22)** (E120) is a natural red dye of anthraquinonic type extracted from the dried females of the insect *Dactylopins coccus* var. *costa* (cochineal), which are widely used as additives in various foods. However, the risk of sensitization to reactive dyes is well established [267]. Carmine has been reported to cause hypersensitivity reactions and has been implicated as an ethiologic agent of occupational asthma, but the allergens involved have not yet been identified [268-271]. Several investigations have reported numerous cases of anaphylactic reaction to carmine [272-275]. Immunoglobulin E-hypersensitivity is a suggested mechanism to explain adverse reactions from carmine-containing products [276,277].

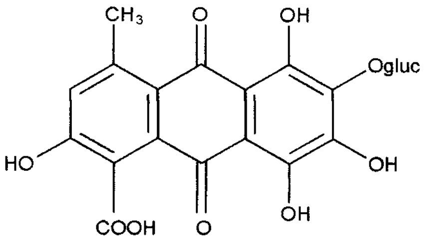


**Fig. (22).** Structure of carmine

Various quinones in the sawdust of commercial timbers are also capable of inducing allergic contact dermatitis and allergic bronchial asthma in

people operating in woodworking industries [278-281]. Similarly, several cases of hairdressers who develop an immediate-type hypersensitivity with urticaria, rhinitis and bronchial asthma on exposure to henna have also been reported [282-284]. Finally, in medicine, pharmacy and technology, the range of interest of naturally-occurring quinones covers their therapeutical as well as their allergological and toxicological properties [285-287].

Other pharmacological properties reported from natural and synthetic quinones, which range from chemically complex phytotherapeutics to simple pure drugs are listed below. For example, Satoh *et al.* [288] studied the effects of 17 kinds of Kampo-formulations and the constitutive crude drugs prescribed for the treatment of peptic ulcer, on H-K-ATPase activity. Among them, the *Rhei* rhizome had a notable inhibitory effect. H-K-ATPase activity was inhibited by sennoside A and B, Fig. **(1)**, two anthraquinonic constituents of this herb. Four compounds of an anthraquinonic type isolated from this medicinal plant (emodin, Fig. **(4)**, rhein, Fig. **(5)**, chrysophanol, Fig. **(3)** and aloe-emodin, Fig. **(2)**) were effective in *Helicobacter pylori* inhibition [289]. Two of these natural anthraquinones, aloe-emodin and rhein, elicited dose-dependent growth inhibition in *Helicobacter pylori* cultures collected from peptic ulcer patients [290,291]. Additionally, rhein has been shown to inhibit indomethacin-induced gastric ulceration in rats [292]. More recently, a novel natural anthraquinone, a benzo[α]anthraquinone which also showed selective anti- *Helicobacter pylori* activity, has been isolated [293].

Several investigations have also demonstrated the hepatoprotective activity of some naturally-occurring anthraquinones. For example, aloe-emodin, Fig. **(2)** one of the active constituents contained in medicinal plants such as *Aloe* spp., appears to have some protective effect on acute liver injury induced by carbon tetrachloride [294]. Other studies reported the hepatoprotective effect of the anthraquinone emodin on hepatic fibrosis in rats. The emodin-treated rats showed improved liver functions and reduced degrees of fibrosis [295].

Several investigations have reported the ability of some natural quinones to be therapeutically useful as hypolipidemic agents. Both experimental and clinical studies have indicated that a novel source of dietary fibre produced from rhubarb is potentially hypolipidemic. The increased excretion of bile acids and induction of cholesterol 7α-hydroxylase activity may account for

the hypocholesteroelemic effect of rhubarb fiber [296]. Anthrones have been shown to be naturally occurring competitive inhibitors of adenosine-triphosphate-citrate lyase, which is a liver enzyme that catalyzes the cytosolic formation of acetyl-CoA. This reaction provides the major source of acetyl-CoA for fatty acids and cholesterol biosynthesis; thus inhibition of this enzyme offers a potentially unique way to control plasma cholesterol and triglyceride levels. Oleynek *et al.* [297] present evidence for 2-chloro-1,3,8-trihydroxy-6-methyl-9-anthrone as a specific and competitive inhibitor of adenosine-triphosphate-citrate lyase, and might prove very useful as a tool for the discovery of selective mechanistically novel hypolipidemic agents.

Several studies have explored the anti-platelet effect produced by naturally occurring quinones. For example, the quinone fraction isolated from *Auxemma oncocalyx* (Allem/Eo) is a reversible and concentration-dependent inhibitor of human platelet aggregation induced by ADP, arachidonic acid, collagen and thrombin [298]. Zhang *et al.* [299] studied the antiplatelet effects of 2-chloro-3-(4-acetophenyl)-amino-1,4-naphthoquinone. This compound significantly inhibited the collagen-, thrombin-, arachidonic acid- and calcium ionophore A23187-induced aggregation of washed human platelets. These investigations suggest that the antiplatelet activity of this naphthoquinone may be mediated by inhibition of cytosolic calcium mobilization, enhancement of AMPc production and inhibition of ATP secretion in activated platelets. A number of antiplatelet quinones, such as two new series of 2-arylmethyl-1,4-benzoquinones are being synthesized [300].

Various antiplatelet drugs, including quinones, are being investigated as potential treatments for cardiovascular diseases because of their ability to prevent excessive platelet aggregation. For example, Kim *et al.* [301] investigated three naphthoquinones (2,3-dimethoxy-1,4-naphthoquinone, menadione and 1,4-naphthoquinone) for their abilities to inhibit platelet aggegation and to deplete glutathione and protein thiols. These results suggest that quinones that deplete glutathione in platelets demonstrate a marked anti-aggregative effect. Yim *et al.* [302] also reported the myocardial protective effect of an anthraquinone-containing extract of *Polygonum multiflorum* L. The more complete myocardial protection afforded for this extract may be related to its activity to sustain the glutathione antioxidant

status under the condition of ischemia-reperfusion-induced oxidative stress. Additionally, protykin, an all-natural standardized extract of trans-reverastrol and the anthraquinone emodin, Fig. **(4)** derived from the dried rhizome of *Polygonum cuspidatum* Sieb & Zucc. can provide cardioprotection, presumably by virtue of its potent free radical scavenging activity [303].

Various constituents isolated from plants, including quinonic compounds, have been shown to possess antihypertensive and spasmolytic activity. For example, naphthoquinones such as juglone, Fig. **(10)**, 7-methyljuglone and plumbagin, Fig. **(11)** showed a calcium-channel blocking activity in an *in vitro* guinea pig papillary muscle model [304]. Saleem *et al.* [305] reported the hypotensive effect of aloe-emodin, Fig. **(2)** and aloin A, two anthraquinonic compounds isolated from *Aloe barbadensis* Miller. One of them, aloe-emodin, has emerged as a potent hypotensive agent. Furthermore, Xu *et al.* [306] observed the inhibition of aloe-emodin on vascular smooth muscle cells in culture after arterial injury.

Recently, Tanaka [307] has reviewed natural quinonic derivatives as candidates for new orally available antidiabetics. The lead asterriquinone B1 was discovered in a fungal extract, and an analog, 2,5-dihydroxy-3-(1-methylindol-3-yl)-6-phenyl-1,4-benzoquinone, was selected by studying various *in vitro* and *in vivo* tests. These studies shed light on the search for new anti-diabetic agents by targeting insulin receptors. Using an ethnomedical-based drug discovery program, two new compounds, pycnanthuquinone A and B were isolated from the African plant *Pcynanthus angolensis* Llamba and were tested using a diabetic mouse model. The antihyperglycemic actions of these compounds were associated with significant decreases in plasma insulin concentrations, suggesting that both lowered glucose by enhancing insulin-mediated glucose uptake. Pycnanthuquinones A and B are the first representatives of a novel terpenoid-type quinone skeleton which may represent a new class of compounds of potential use in the treatment of type 2 diabetes [308,309]. It has also been suggested that glucose transport activity is an important modulator of cellular glucose metabolism in messangial cells. Zhu *et al.* [310] demonstrated that rhein, Fig. **(5)**, a natural anthraquinone, could ameliorate the metabolic derangement of the human glucose transporter 1 gene by decreasing glucose uptake. These findings have led us closer to the identification of therapeutic

approaches to abort glucose transporter 1 overexpression in diabetic nephropathy.

Several investigations have reported the ability of some natural quinones to be therapeutically useful in the treatment of asthma. The natural anthraquinone chrysophanol-8-O-β-D-glucopyranoside has potent inhibitory activity on hyaluronidase, a enzyme released from mast cells and passive cutaneous anaphylaxis reactions [311]. Cheng *et al.* [312] described the inhibitory effect of 9,10-anthraquinone 2-carboxylic acid on immunoglobulin E-mediated passive cutaneous anaphylaxis reaction. Furthermore, these compounds are promising for treating allergic diseases with chronic and severe pruritus. Lawsone, Fig. **(12)**, 2-hydroxy-3-(2-hydroxyethyl)-1,4-naphthoquinone and 2,2'-methylenebis(3-hydroxy)-1,4-naphthoquinone inhibited scratching behavior in mice with established dermatitis, and they may be effective for the prevention and treatment of atopic dermatitis [313,314].

## PLANT CELL CULTURES AS SOURCES OF QUINONES

Cultured plant cells may serve as sources of the various quinones characteristic of intact plants; in addition, new quinones have been produced in plant cell cultures which are not formed in the corresponding parent plants, while sometimes the cultured cells lack the ability to produce easily detectable amounts of natural quinones. For example, callus tissue of *Aloe barbadensis* grown in the dark produced two new tetrahydroanthracene glucosides: 3,4-dihydro-2,4,8,9-tetrahydroxy-6-methyl-anthracenone-4-glucopyranoside and 3,4-dihydro-2-methoxy-4,8,9-trihydroxy-6-methyl-anthracenone-4-glucopyranoside [315]. The cell suspension of *Cassia angustifolia* (senna) was found to produce chrysophanol, physcion and rhein as anthraquinones and several bianthrones, although no sennosides, strong purgative agents in senna were detected in the cell cultures [316].

Several investigations reported the stimulation of anthraquinone production in suspension cultures of *Cassia acutifolia* Del. by salt stress. Pot culture experiments were conducted using NaCl concentrations to assess their impact on the growth and metabolic changes in this plant [317]. Suspension cultures of *Cassia acutifolia* were also established by transferring callus

tissues derived from root, hypocotyl and cotyledon explants onto medium suppplemented with kinetin and containing increased levels of NaCl. The stress induced by salt NaCl raised anthraquinone content and reduced growth of cultures. Furthermore, the salt stress tended to affect more drastically the productivity of anthraquinones in hypocotyl and cotyledon cell cultures than in root cultures [318,319].

With a few exceptions, the characteristic problem of cultivation of plant explants in *in vitro* cultures is a low production of secondary metabolites by these cultures. One of the methods which can achieve an increase in the production of natural substances in *in vitro* cultures, is elicitation of cell cultures with biotic elicitors. For example, hairy root cultures of *Cassia obtusifolia* L. clones transformed with *Agrobacterium rhizogenes* strain 9402 were established to investigate anthraquinone production. It was found that changes of the elements in the culture medium and the addition of rare earth element $Eu^{3+}$ can greatly influence the contents of free anthraquinones in the hairy root [320].

Hairy root culture of the medicinal plant *Rheum palmatum* was established by genetic transformation with *Agrobacterium rhizogenes* and the effects of various media with different pH on growth of the hairy roots and biosynthesis of free anthraquinones were investigated. Cultivation with auxin activated hairy root growth but inhibited the biosynthesis of free anthraquinones [321]. Tomova and Dusek [322], investigated the effect of linoleic acid on the production of anthracene derivatives in *Rheum palmatum* culture *in vitro*. In the elicitation of the culture, the highest response in the production of anthraquinones was observed in linoleic acid in a concentration of 1 mg/ml. Furthermore, the effect of the biotic elicitor *Pseudomonas aeruginosa* in the form of a homogenate and an aqueous suspension of dead cells on the production of anthracene derivatives by the tissue culture of *Rheum palmatum* of a different age and origin was examined. The culture, newly derived from the root of the intact plant, responded to elicitation more sensitively than the culture derived from the seed; nevertheless the content of anthracene derivatives was lower than in the several year old culture [323,324]. More recently, Sepehr and Ghorbanli [325] have studied the effect of nutritional factors on the formation of anthraquinones in callus cultures of *Rheum ribes* L., one of the most important varieties of rhubarb in Asiatic

regions. These studies have demonstrated that the growth rate of callus declined with the increased rate of secondary metabolite production. Kovacevic *et al.* [326] investigated the organogenesis and somatic embryogenesis in cotyledon cultures of *Rhamnus catharticus* L. and in zygotic embryo. Production of physcion derivatives was significantly increased in embryogenic calluses compared to zygotic embryos, but the emodin content was lower than in zygotic embryos.

Plants and their derived cells and tissue cultures in the family Rubiaceae accumulate a number of anthraquinones [327]. One of the aims of the research into the formation of secondary compounds by plant cell cultures is their industrial-scale production. To enhance the productivity of anthraquinone colorants during madder (*Rubia tinctorum*) cell cultures, Shim *et al.* [328] investigated the effects of permeabilizing agents on the production of anthraquinones. Addition of Tween 80 increased the total and released concentrations of anthraquinones. In addition, anthraquinone production was increased by simultaneous use of Tween 80 and chitosan. The effects of auxines and different conditions of illumination on the growth rates of tissue cultures of *Rubia tinctorum* were also studied with regard to the production of anthraquinones. The cultivation of selected callus in medium containing kinetin in the dark showed the best result for the production of anthraquinones, and in the light, the best growth of cultures [329]. More recently, Boka *et al.* [330] have investigated the effect of two elicitor types prepared from three different fungi on the alizarin content in *Rubia tinctorum* suspension culture. These studies suggest that *Botrytis* elicitor are the most effective. Elicitation of genetically transformed madder roots with methyl jasmonate did not influence the growth of their culture, but resulted in an increase in the anthraquinone content without alteration in the qualitative pigment composition [331]. Similarly, an *Agrobacterium rhizogenes*-mediated transformation system for *Rubia peregrina* L. has been established by co-cultivation of callus cultures or by direct infection of explants with this elicitor. The accumulation of total anthraquinones in transformed roots was found to be approximately two-fold higher than that found in one-year-old field-grown roots. Alizarin was found to be the major anthraquinone in transformed root cultures [332].

Other cultured plant cells may serve as sources of new anthraquinones which are not formed in the corresponding parent plants. The chemical investigation on the cell suspension culture of *Morinda elliptica* yielded eight anthraquinones, two of them, anthragallol-1,2-dimethyl ether and purpurin-1-methyl ether, have not been isolated from the original plant [333]. The effects of medium strategy and mode of operation on growth and anthraquinone production of *Morinda elliptica* cell suspension cultures are described [334-336]. *Morinda citrifolia* (Noni) cell suspension cultures are also capable of accumulating high levels of anthraquinones [337].

Other studies have also demonstrated a remarkable difference in constituents between wild plants and callus tissue or cultured cells. For example, tissue and cell culture of *Ophiorrhiza pumila* L. revealed the presence of 11 anthraquinones, including two new ones not present in the parent plant [338]. New compounds of anthraquinone type were also isolated from *Isoplexis isabelliana* (Webb) Masf, *Fusarium oxysporum* L. and *Cruciata glabra* (L.) Ehrend cell suspension cultures [339-341].

A suspension culture of *Cinchona robusta* How, which under normal culture does not produce anthraquinones, produces a range of these compounds after elicitation. Eight new anthaquinones (robustaquinones A-H) were identified, in addition to two known anthraquinones [342,343]. Treatment of *Cinchona robusta* cell suspension culture with a homogenate of *Phytophthora cinnamomi* resulted in cessation of growth and a rapid induction of the biosynthesis of anthraquinones. The strongest induction of anthraquinone production was obtained when the elicitor was added in the early growth phase of the growth cycle [344].

Cultured plant cells may also serve as sources of various naphthoquinones and benzoquinones, whether or not they are present in the whole plants. For example, suspension cultures of *Panax ginseng* C. A. Meyer were treated with either an elicitor preparation from the phytopathogenic *Botrytis cinerea* or a yeast elicitor preparation, and the accumulation of a new compound (2,5-dimethoxy-1,4-benzoquinone), which was not detected in non-elicited cultures, was observed [345]. Induction of naphthoquinone formation in *Impatients balsamina* cell cultures was achieved by using parent plants yielding high levels of 2-methoxy-1,4-naphthoquinone as initiated explants. The cell cultures were capable of producing two naphthoquinones, lawsone

and an unknown compound, which was more polar than lawsone [346]. From the shoots of *Drosera gigantea* L. propagated under *in vitro* cultures, the rare naphthoquinone glucosides droserone (3,5-dihydroxy-2-methyl-1,4-naphthoquinone) and 8-hydroxy-droserone 5-glucosides, together with free naphthoquinones droserone, 8-hydroxy-droserone and plumbagin were isolated. Of the other naphthoquinones typical for the familiy Droseraceae, only hydroxyplumbagin glucoside could be detected, whereas the presence of 7-methyl-juglone and rossoliside (7-methyl-hydrojuglone glucoside) was excluded [347]. Droserone contents are also reported in *in vitro* cultured plants and cell suspensions of *Drosera capensis* L., *Dionaea muscipula* Ellis ex L. and *Triphyophyllum peltatum* Hutch [348-350]. The methanolic extracts of *Drosera rotundifolia* L. and *Drosera spathulata* H. R. obtained by *in vitro* micropropagation, yielded the new pigment 2-methyl-naphthazarin 5-glucoside [351].

Successful commercialisation of the formation of the secondary compounds by plant cell cultures has been achieved by the production of shikonin pigments from plant cell cultures of *Lithospermun erythrorhizon* S. Hwang by Mitsui Petrochemical Industries Ltd. Several studies have investigated factors influencing shikonin production in cultured cells. For example, studies were conducted with a BK-39 callus culture of *Lithospermun erythrorhizon*, which produced seven shikonin derivatives. Selected BK-39 cultures produced almost the same profile of shikonin naphthoquinones as the initial culture [352].

Furthermore, use of *Hypericum perforatum* has increased recently due to the pharmaceutical potential of the naphthodianthrone hypericin found in its leaves. Hypericin has been reported to effect a natural treatment for mild and moderate depression. Bais *et al.* [353] have recently developed a novel cell culture system for *in vitro* growth and production of this species, suggesting a possible technology for large-scale production of hypericin. Light and dark conditions, with cell aggregate size, played important roles in growth and hypericin production in cell suspension cultures. Previously, Kirakosyan *et al.* [354] investigated shoot organ cultures from callus derived from anthers of *Hypericum perforatum* flowers and the effect of several elicitors on the production of hypericins. Pectin and β-1,3-glucan slightly stimulated pseudohypericin production, but had no effect on hypericin production.

Finally, the stimulating effect of cork pieces on hypericin and pseudophypericin production in cells of shoots regenerated from the callus cultures of *Hypericum perforatum* has also been reported [355].

## ABBREVIATIONS

DMNQ = 5,8-Dimethoxy-1,4-naphthoquinone
TPA = 12-*O*-Tetradecanoylphorbol-13-acetate
TNF-α = Tumor necrosis factor-α
LPS = Lipopolysaccharide
NO = Nitric oxide
PGs = Prostaglandins
COX-2 = Cyclooxygenase-2
iNOS = Inducible nitric oxide synthase
JNK = Jun N-terminal kinase
HIV = Human immunodeficiency virus
AIDS = Acquired immunodeficiency syndrome
RNase H = Ribonuclease H
RT = Reverse transcriptase
HSV-1 = Herpes simplex virus type I
HCMV = Human cytomegalovirus
5-HT = Serotonin
NA = Noradrenaline
DA = Dopamine
GABA = γ-Aminobutyric acid

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## REFERENCES

[1] Wang, H.J.; Gloer, K.B.; Gloer, J.B.; Scott, J.A.; Malloch, D.; *J. Nat. Prod*, **1997,** *60,* 629-631.
[2] Joshi, S.P.; *J. Med. Aromat. Plant Sci*, **1998,** *20,* 768-773.

[3] Medentsev, A.G.; Akimenko, V.K.; *Phytochemistry*, **1998,** *47,* 935-959.

[4] Kulanthaivel, P.; Perun, T.J.; Belvo, M.D.; Strobel, R.J.; Paul, D.C.; Williams, D.C.; *J. Antibiot*, **1999,** *52,* 256-262.

[5] Lysak, E.I.; Demina, G.R.; Matveeva, E.V.; Ogrel, O.D.; Trutko, S.M.; Ostrouskii, D.N.; *Microbiology*, **1999,** *68,* 1-9.

[6] Dagne, E.; Bisrat, D.; Viljoen, A.; Vanwyk, B.E. ; *Curr. Org. Chem*, **2000,** *4,* 1055-1078.

[7] Viljoen, A.M.; Van Wykand, B.E.; Newton, L.E.; *Biochem. Syst. Ecol*, **2001,** *29,* 53-67.

[8] Nishida, F.; Nishijima, M.; Mochida, K.; Sano, H.; Nomura, N.; Sako, Y.; Marugama, T.; *FEMS Microbiol. Lett*, **1999,** *174,* 339-346.

[9] Sugimoto, N.; Goda, Y.; Suzuki, J.; Kuroyanagi, M.; Yamada, T.; Yoshihira, K.; Maitani, T.; *Nat. Med*, **1998,** *52,* 135-139.

[10] Yezerski, A.; Gilmor, T.P.; Stevens, L. *Physiol. Biochem. Zool*, **2000,** *73,* 192-199.

[11] Yang, L.; Akao, T.; Kobashi, K.; Hattori, M.; *Biol. Pharm. Bull*, **1996,** *19,* 705-709.

[12] Yang, L.; Akao, T.; Kobashi, K.; Hattori, M.; *Biol. Pharm. Bull*, **1996,** *19,* 701-704.

[13] Akao, T.; Che, Q.M.; Kobashi, K.; Hattori, M.; Namba, T.; *Biol. Pharm. Bull*, **1996,** *19,* 136-138.

[14] Lemli, J.; *Ann. Gastroenterol. Hepatol*, **1996,** *32,* 109-112.

[15] Yagi, T.; Yamauchi, K.; Kuwano, S.; *J. Pharm. Pharmacol*, **1997,** *49,* 22-25.

[16] Maity, T.K.; Mandal, S.C.; Bhakta, T.; Pal, M.; Saha, B.P.; *Phytother. Res*, **2001,** *15,* 459-460.

[17] Yagi, T.; Yamauchi, K. ; *J. Pharm. Pharmacol*, **1999,** *51,* 93-95.

[18] Kuo, D.H.; Kuo, S.C.; Cheng, J.T.; *J. Pharm. Pharmacol*, **2000,** *52,* 839-841.

[19] Kamei, H.; Koide, T.; Kojima, T.; Hashimoto, Y.; Hasegawa, M.; *Cancer Biother. Radiopharm*, **1998,** *13,* 185-188.

[20] Lee, H.Z.; *Br. J. Pharmacol*, **2001,** *134,* 11-20.

[21] Lee, H.Z.; Hsu, S.L.; Liu, M.C.; Wu, C.H.; *Eur. J. Pharmacol*, **2001,** *431,* 287-295.

[22] Shi, Y.Q.; Fukai, T.; Sakagami, H.; Karoda, J.; Miyaoka, R.; Tamura, M.; Yoshida, N.; Nomura, T.; *Anticancer Res*, **2001,** *21,* 2847-2853.

[23] Sun, M.Z.; Sakakibura, H.; Ashida, H.; Danno, C.; Kanazawa, K.; *Biosci. Biotechnol. Biochem*, **2000,** *64,* 1373-1378.

[24] Kuo, Y.C.; Tsai, W.J.; Meng, H.C.; Chen, W.P.; Yang, L.Y.; Lin, C.Y.; *Life Sci*, **2001,** *68,* 1271-1286.

[25] Pecere, T.; Gazzola, M.V.; Mucignat, C.; Parolin, C.; Dalla-Vecchia, F.; Cavaggioni, A.; Basso, G.; Diaspro, A.; Salvato, B.; Carli, M.; Palu, G.; *Cancer Res*, **2000,** *60,* 2800-2804.

[26] Wasserman, L.; Avigad, S.; Beery, E.; Nordenberg, J.; Fenig, E.; *Am. J. Dermatopathol*, **2002,** *24,* 17-22.

[27] Grimaudo, S.; Tolomeo, M.; Gancitano, R.A.; D' Alessandro, N.; Aiello, E.; *Oncol. Rep*, **1997,** *4,* 341-343.

[28] Shimpo, K.; Chihara, T.; Beppu, H.; Ida, C.; Kaneko, T.; Nagatsu, T.; Kuzuya, H.; *Phytother. Res*, **2001,** *15,* 705-711.

[29] Fahim, F.A.; Esmat, A,Y.; Mady, E.A.; Amin, M.A.; *J. Tumour Marker Oncol*, **1997,** *12,* 63-64.

[30] Fahim, F.A.; Esmat, A,Y.; Mady, E.A.; Amin, M.A.; *J. Tumour Marker Oncol*, **1997,** *12,* 127-134.

[31] Fahim, F.A.; Esmat, A,Y.; Mady, E.A.; Amin, M.A.; *Dis. Markers*, **1997,** *13,* 183-193.

[32] El Metenawy, W.H.; Esmat, A.Y.; *Cancer Mol. Biol*, **1996,** *3,* 851-864.

[33] Ali, A.M.; Ismail, N.H.; Mackeen, M.M.; Yazan, L.S.; Mohamed, S.M.; Ho, A.S.H.; Lajis, N.H.; *Pharm. Biol*, **2000,** *38,* 298-301.

[34] Edenharder, R.; Speth, C.; Decker, M.; Platt, K.L.; *Z. Lebens. Untersuch. Forsch. A Food Res. Technol*, **1998,** *207,* 464-471.

[35] Wei, B.L.; Wu, S.H.; Chung, M.I.; Won, S.J.; Lin, C.N.; *Eur. J. Med. Chem*, **2000,** *35,* 1089-1098.

[36] Jin, G.Z.; You, Y.J. Ahn, B.E.; *Bioorg. Med. Chem. Lett*, **2001,** *11,* 1473-1476.

[37] Jin, G.Z.; You, Y.J.; Kim, Y.; Nam, N.H.; Ahn, B.E.; *Eur. J. Med. Chem*, **2001,** *36,* 361-366.

[38] Cardia, M.C.; Begala, M.; De Loga, A.; Maccioni, E.; *Farmaco*, **2001,** *56,* 549-554.

[39] Zagotto, G.; Supino, R.; Favini, E.; Moro, S.; Palumbo, M.; *Farmaco*, **2000,** *55*, 1-5.

[40] Yoo, K.H.; Yoon, E.Y.; Park, Y.Y.; Park, S.W.; Lee, C.O.; Lee, W.K.; Chi, D.Y.; Kim, D.J.; *Bull. Korean Chem. Soc*, **2001,** *22*, 1067-1068.

[41] Skibo, E.B.; Xing, C.G.; Dorr, R.T.; *J. Med. Chem*, **2001,** *44*, 3545-3562.

[42] Huang, H.S.; Hwang, J.M.; Jen, Y.M.; Lin, J.J.; Lee, K.Y.; Shi, C.H.; Hsu, H.C.; *Chem. Pharm. Bull*, **2001,** *49*, 969-973.

[43] Cairns, D.; Michalitsi, E.; Jenkins, T.C.; Mackay, S.P.; *Bioorg. Med. Chem*, **2002,** *10*, 803-807.

[44] Li, C.J.; Wang, C.; Pardee, A.B.; *Cancer Res*, **1995,** *55*, 3712-3715.

[45] Krishnan, P.; Bastow, K.F.; *Biochem. Pharmacol*, **2000,** *60*, 1367-1379.

[46] Dolan, M.E.; Frydman, B.; Thompson, C.B.; Diamond, A.M.; Garbiras, B.J.; Safa, A.R.; Beck, W.T.; Marton, L.J.; *Anticancer Drugs*, **1998,** *9*, 437-448.

[47] Don, M.J.; Chang, Y.H.; Chen, K.K.; Ho, L.K.; Chau, Y.P.; *Mol. Pharmacol*, **2001,** *59*, 784-794.

[48] Chau, Y.P.; Shiah, S.G.; Don, M.J.; Kuo, M.L.; *Free Radic. Biol. Med*, **1998,** *24*, 660-670.

[49] Li, Y.Z.; Li, C.J.; Yu, D.H.; Pardee, A.B.; *Mol. Med*, **2000,** *6*, 1008-1015.

[50] Weller, M.; Winter, S.; Smidt, C.; Esser, P.; Fontana, A.; Dichgans, J.; Groscurth, P.; *Int. J. Cancer*, **1997,** *73*, 707-714.

[51] Pink, J.J.; Planchon, S.M.; Tagliarino, C.; Varnes, M.E.; Siegel, D.; Boothman, D.A.; *J. Biol. Chem*, **2000,** *275*, 5416-5424.

[52] Lai, C.C.; Liu, T.J.; Ho, L.K.; Don, M.J.; Chau, Y.P.; *Histol. Histopathol*, **1998,** *13*, 89-97.

[53] Krishnan, P.; Bastow, K.F.; *Cancer Chem. Pharmacol*, **2001,** *47*, 187-198.

[54] Li, C.J.; Li, Y.Z.; Pinto, A.V.; Pardee, A.B.; *Proc. Natl. Acad. Sci. USA*, **1999,** *96*, 13369-13374.

[55] Frydman, B.; Marton, L.J.; Sun, J.S.; Neder, K.; Witiak, D.T.; Liu, A.A.; Wang, H.M.; Mao, Y.; Wu, H.Y.; Sanders, M.M.; Liu, L.F.; *Cancer Res*, **1997,** *57*, 620-627.

[56] Noda, Y.; Kawazoe, Y.; Hakura, A.; *Biol. Pharm. Bull*, **1997,** *20,* 1250-1256.

[57] Nair, V.; Treesa, P.M.; *Tetrahedron Lett*, **2001,** *42,* 4549-4551.

[58] Di Chenna, P.H.; Benedetti-Doctorovich, V.; Baggio, R.F.; Garland, M.T.; Burton, G.; *J. Med. Chem*, **2001,** *44,* 2486-2489.

[59] Plyta, Z.F.; Li, T.; Papageorgiov, V.P.; Mellidis, A.S.; Assimopoulov, A.N.; Pitsinos, E.N.; Couladouros, E.A.; *Bioorg. Med. Chem. Lett*, **1998,** *8,* 3385-3390.

[60] Hisa, T.; Kimura, Y.; Takada, K.; Suzuki, F.; Takigawa, M.; *Anticancer Res*, **1998,** *18,* 783-790.

[61] Naresh, R.A.R.; Udupa, N.; Devi, P.U.; *J. Pharm. Pharmacol*, **1996,** *48,* 1128-1132.

[62] Munday, R.; Munday, C.M.; *Planta Med*, **2000,** *66,* 399-402.

[63] Karczewski, J.M.; Peters, J.G.P.; Noordhaek, J.; *Biochem. Pharmacol*, **1999,** *57,* 27-37.

[64] Osada, S.; Carr, B.I.; *J. Hepatol*, **2001,** *34,* 676-682.

[65] Huang, M.H.; Wu, S.N.; Chen, C.P.; Shen, A.Y.; *Life Sci*, **2002,** *70,* 1185-1203.

[66] You, Y.J.; Ahn, B.Z.; *Arch. Pharmacal. Res*, **1998,** *21,* 738-743.

[67] Chae, G.H.; Song, G.Y.; Kim, Y.; Cho, H.; Sok, D.E.; Ahn, B.Z.; *Arch. Pharmacal. Res*, **1999,** *22,* 507-514.

[68] You, Y.G.; Zheng, X.G.; Yong, R.; Ahn, B.Z.; *Arch. Pharmacal. Res*, **1998,** *21,* 595-598.

[69] Song, G.Y.; Kim, Y.; You, Y.J.; Cho, H.; Ahn, B.Z.; *Arch. Pharmacal. Res*, **2001,** *24,* 190-193.

[70] Kim, Y.; You, Y.J.; Ahn, B.Z.; *Arch. Pharm*, **2001,** *334,* 318-322.

[71] Wipf, P.; Hopkins, T.D.; Jung, J.K.; Rodriguez, S.; Birminghan, A.; Southwick, E.C.; Lazo, J.S.; Powis, G.; *Bioorg. Med. Chem. Lett*, **2001,** *11,* 2637-2641.

[72] Gokhale, N.; Padhye, S.; Newton, C.; Pritchard, R.; *Met. Based Drugs*, **2000,** *7,* 121-128.

[73] Wu, C.M.; Johnson, R.K.; Mattern, M.R.; Wong, J.C.; Kingston, D.G.I.; *J. Nat. Prod*, **1999,** *62,* 963-968.

[74] Pan, J.; Simamura, E.; Koyama, J.; Shimada, H.; Hirai, K.I.; *Cancer Detect. Prev*, **2000,** *24,* 266-274.

[75] Itoigawa, M.; Ito, C.; Tan, H.T.W.; Okuda, M.; Tokuda, H.; Nishino, H.; Furukawa, H.; *Cancer Lett*, **2001,** *174,* 135-139.
[76] Lee, Y.R.; Kim, B.S.; *Synth. Commun*, **2001,** *31,* 381-386.
[77] Zupko, I.; Kamuhabwa, A.R.; D'Hallewin, M.A.; Baert, L.; De Witte, P.A.M.; *Int J. Oncol*, **2001,** *18,* 1099-1105.
[78] Chen, B.; Zupko, I.; De Witte, P.A.; *Int. J. Oncol*, **2001,** *18,* 737-742.
[79] Uzdensky, A.B.; Ma, L.W.; Iani, V.; Hjortland, G.O.; Steen, H.B.; Moan, J.; *Laser Med. Sci*, **2001,** *16,* 276-283.
[80] Schempp, C.M.; Simon-Haarhaus, B.; Termeer, C.C.; Simon, J.C.; *FEBS Lett*, **2001,** *493,* 26-30.
[81] Schempp, C.M.; Simon-Haarhaus, B.; Simon, J.C.; *Planta Med*, **2002,** *68,* 171-173.
[82] Agostinis, P.; Vantieghem, A.; Merlevede, W.; De Wite, P.A.M.; *Int. J. Biochem. Cell Biol*, **2002,** *34,* 221-241.
[83] Chen, B.; Roskams, T.; Xu, Y.; Agostinis, P.; De Witte, P.A.M.; *Int. J. Cancer*, **2002,** *98,* 284-290.
[84] Le Lain, R.; Nicholls, P.J.; Smith, H.J.; Maharlovie, F.M.; *J. Enzyme Inhib*, **2001,** *16,* 35-45.
[85] Ward, T.H.; Butler, J.; Shahbakhti, H.; Richards, J.T.; *Biochem. Pharmacol*, **1997,** *53,* 1115-1121.
[86] Van Gorkom, B.A.P.; Karrenbeld, A.; Limburg, A.J.; Kleibeuker, J.H.; *Z. Gastroenterol*, **1998,** *36,* 13-18.
[87] Van Gorkom, B.A.P.; De Vries, E.G.E.; Karrenbeld, A.; Klebeuker, J.H.; *Aliment. Pharmacol. Ther*, **1999,** *13,* 443-452.
[88] Mereto, E.; Ghia, M.; Brambilla, G.; *Cancer Lett*, **1996,** *101,* 79-83.
[89] Brusick, D.; Mengs, U.; *Environ. Mol. Mut*, **1997,** *29,* 1-9.
[90] Mueller, S.O.; Schmitt, M.; Dekant, W.; Stopper, H.; Schlatter, J.; Schreier, P.; Lutz, W.K.; *Food Chem. Toxicol*, **1999,** *37,* 481-491.
[91] Mueller, S.O.; Lutz, W.K.; Stopper, H.; *Mut. Res*, **1998,** *414,* 125-129.
[92] Mueller, S.O.; Eckert, I.; Lutz, W.K.; Stopper, H.; *Mut. Res. Genet. Toxicol*, **1996,** *371,* 165-173.
[93] Mueller, S.O.; Stopper, H.; *Biochim. Biophys. Acta*, **1999,** *1428,* 406-414.
[94] Mueller, S.O.; Stopper, H.; Dokant, W.; *Drug Metab. Dispos*, **1998,** *26,* 540-546.

[95] Longo, V.; Amato, G.; Salvetti, A.; Gervasi-Pier, G.; *Chem. Biol. Interact*, **2000,** *126,* 63-77.
[96] Wang, H.W.; Chen, T.L.; Yang, P.C.; Ueng, T.H.; *Drug Metab. Dispos*, **2001,** *29,* 1229-1235.
[97] Tanaka, T.; Kohno, H.; Murakami, M.; Shimada, R.; Kagami, S.; *Oncol. Rep*, **2000,** *7,* 501-508.
[98] Westendorf, J.; Pfau, W.; Schulle, A.; *Carcinogenesis*, **1998,** *19,* 2163-2168.
[99] Marec, F.; Kollarova, I.; Jegorov, A.; *Planta Med*, **2001,** *67,* 127-131.
[100] Battalora, M.S.J.; Johnston, D.A.; Di Giovannia, J.; *Cancer Lett*, **1995,** *98,* 19-25.
[101] Strickland, F.M.; Muller, H.K.; Stephens, L.C.; Bucana, C.D.; Donawho, C.K.; Sun, Y.; Pelley, R.P.; *Photochem. Photobiol*, **2000,** *72,* 407-414.
[102] Mukhopadhyay, M.J.; Saha, A.; Dutta, A.; De, B.; Mukherjee, A.; *Food Chem. Toxicol*, **1998,** *36,* 937-940.
[103] Martínez, A.; Urios, A.; Blanco, M.; *Mut. Res*, **2000,** *467,* 41-53.
[104] Guerra, M.O.; Mazoni, A.S.; Brandao, M.A.; Peters, V.M.; *Rev. Bras. Biol*, **2001,** *61,* 171-174.
[105] Cuellar, M.J.; Giner, R.M.; Recio, M.C.; Mañez, S.; Rios, J.L.; *Fitoterapia*, **2001,** *72,* 221-229.
[106] Wei, B.L.; Lu, C.M.; Tsao, L.T.; Wang, J.P.; Lin, C.N.; *Planta Med*, **2001,** *67,* 745-747.
[107] Kuo, Y.C.; Meng, H.C.; Tsai, W.J.; *Inflamm. Res*, **2001,** *50,* 73-82.
[108] Spencer, C.M.; Wilde, M.I.; *Drugs*, **1997,** *53,* 98-106.
[109] Moldovan, F. ; Pelletier, J. ; Jolicoeur, F. ; Cloutier, J. ; Martel-Pelletier, J. ; *Osteoarthritis Cartilage*, **2000,** *8,* 186-196.
[110] Carney, S.L.; Hicks, C.A.; Tree, B.; Broadmore, R.J.; *Inflamm. Res*, **1995,** *44,* 182-186.
[111] Carney, S.L.; *Osteoarthritis Cartilage*, **1996,** *4,* 251-261.
[112] Pelletier, J.P.; Mineau, F. ; Fernandes, J.C. ; Duval, N. ; Martel-Pelletier, J. ; *J. Rheumatol*, **1998,** *25,* 2417-2424.
[113] Chen, Y.C.; Yang, L.L.; Lee, T.J.F. ; *Biochem. Pharmacol*, **2000,** *59,* 1445-1457.

[114] Bennett, B.L.; Sasaki, D.T.; Murray, B.W.; O'Leary, E.C.; Sakata, S.T.; Xu, W.; Leisten, J.C.; Motiwala, A.; Pierce, S.; Satoh, Y.; Bhagwat, S.S.; Manning, A.M.; Anderson, D.W.; *Proc. Natl. Acad. Sci. USA*, **2001,** *98,* 13681-13686.
[115] Reddi, K.; Henderson, B.; Meghji, S.; Wilson, M.; Poole, S.; Hopper, C.; Harris. M.; Hodges, S.J.; *Cytokine*, **1995,** *7,* 287-290.
[116] Battistutta, R.; Sarno, S.; De Moliner, E.; Papinutto, E.; Zanotti, G.; Pinna, L.A.; *J. Biol. Chem*, **2000,** *275,* 29618-29622.
[117] Brauers, G.; Edrada, R.A.; Ebel, R.; Proksch, P.; Wray, V.; Berg, A.; Grafe, U.; Schachtele, G.; Totzke, F.; Finkenzeller, G.; Marme, D.; Kraus, J.; Munchbach, M.; Michel, M.; Bringmann, G.; Schaumann, K.; *J. Nat. Prod*, **2000,** *63,* 739-745.
[118] Bezakova, L.; Psenak, M.; Kartnig, T.; *Pharmazie*, **1999,** *54,* 711.
[119] Muller, K.; Breu, K.; Reindl, H.; *Eur. J. Med. Chem*, **2001,** *36,* 179-184.
[120] Wurm, G.; Probst, R.; Schwandt, S.; *Pharmazie*, **2001,** *56,* 527-533.
[121] Amano, T.; Terao, S.; Imada, I.; *Biol. Pharm. Bull*, **1995,** *18,* 779-781.
[122] Lien, J.C.; Huang, L.J.; Wang, J.P.; Teng, C.M.; Lee, K.H.; Kuo, S.C.; *Chem. Pharm. Bull*, **1996,** *44,* 1181-1187.
[123] Lien, J.C.; Huang, L.J.; Wang, J.P.; Teng, C.M.; Lee, K.H.; Kuo, S.C.; *Bioorg. Med. Chem*, **1997,** *5,* 2111-2120.
[124] Wurm, G.; Schwandt, S.; *Pharmazie*, **1999,** *54,* 487-490.
[125] Scheibler, P.; *Pharmazie*, **1997,** *52,* 411-412.
[126] Yen, G.C.; Duh, P.D.; Chuang, D.Y.; *Food Chem*, **2000,** *70,* 437-441.
[127] Yuan, Z.; Gao, R.; *Pharm. Pharmacol. Lett*, **1997,** *7,* 9-12.
[128] Yen, G.C.; Chen, H.W.; Duh, P.D.; *J. Agric. Food Chem*, **1998,** *46,* 820-824.
[129] Choi, J.S.; Chung, H.Y.; Jung, H.A.; Park, H.J.; Yokozawa, T.; *J. Agric. Food Chem*, **2000,** *48,* 6347-6351.
[130] Sheu, S.Y.; Chiang, H.C.; *Anticancer Res*, **1997,** *17,* 3293-3297.
[131] Storozhok, N.M.; Drulle, A.Y.; Login, Y.Y.; Dregeris, Y.Y.; Khrapova, N.G.; Berlakova, E.B.; *Vopr. Med. Khim*, **1995,** *41,* 16-21.
[132] Miyadera, H.; Kano, K.; Miyoshi, H.; Ishii, N.; Hekimi, S.; Kita, K.; *FEBS Lett*, **2002,** *512,* 33-37.

[133] Kumbhar, A.S.; Padhye, S.B.; Jitender, R.K.; *Met. Based Drugs*, **1997**, *4*, 279-285.
[134] Lebedev, A.V.; Levitskaya, E.L.; Tikhonova, E.V.; Ivanova, M.V.; *Biochemistry*, **2001**, *66*, 885-893.
[135] Gao, D.; Kakuma, M.; Oka, S.; Sugino, K.; Sakurai, H.; *Bioorg. Med. Chem*, **2000**, *8*, 2561-2569.
[136] Hunt, E.J.; Lester, C.E.; Lester, E.A.; Tackett, R.L.; *Life Sci*, **2001**, *69*, 181-190.
[137] Mori, K.; Ushio, T.; Okamoto, T.; Kishi, T.; Sayo, H.; *Biol. Pharm. Bull*, **1998**, *21*, 293-296.
[138] Giorgini, E.; Tommasi, G.; Stipa, P.; Tosi, G.; Littarru, G.; Greci, L.; *Free Radic. Res*, **2001**, *35*, 63-72.
[139] Wiegrebe, W.; Muller, K.; *Skin Pharmacol*, **1995**, *8*, 1-24.
[140] Muller, K.; Huang, H.S.; *Chin. Pharm. J. Taiwan*, **1996**, *48*, 337-354.
[141] Muller, K.; *Gen. Pharmac*, **1996**, *27*, 1325-1335.
[142] Lange, R.W.; Germolec, D.R.; Foley, J.F.; Luster, M.I.; *J. Leukoc. Biol*, **1998**, *64*, 170-176.
[143] Muller, K.; Reindl, H.; Breu, K. ; *Bioorg. Med. Chem. Lett*, **2001**, *11*, 47-50.
[144] Muller, K.; Huang, H.S.; Wiegiebe, W.; *J. Med. Chem*, **1996**, *39*, 3132-3138.
[145] Huang, H.S.; *Chin. Pharm. J*, **1998**, *50*, 267-276.
[146] Muller, K.; Reindl, H.; Breu, K.; *J. Med. Chem*, **2001**, *44*, 814-821.
[147] Muller, K.; Sellmer, A.; Wiegebe, W.; *J. Nat. Prod*, **1999**, *62*, 1134-1136.
[148] Hussein, G.; Miyashiro, H.; Nakamura, N.; Hatori, M.; Kakiuchi, N.; Shimotohno, K.; *Phytother. Res*, **2000**, *14*, 510-516.
[149] Kim, T.G.; Kang, S.Y.; Jung, K.K.; Kang, J.H.; Lee, E.; Han, H.W.; Kim, S.H.; *Phytother. Res*, **2001**, *15*, 718-720.
[150] Jacobson, J.; Feinman, L.; Liebes, L.; Ostrow, N.; Koslowski, V.; Tobia, A.; Cabana, B.E.; Lee, D.H.; Spritzler, J.; Prince, A.M.; *Antimicrob. Agents Chemother*, **2001**, *45*, 517-524.
[151] Schmitt, A.C.; Ravazzolo, A.P.; Von Poser, G.L.; *J. Ethnopharmacol*, **2001**, *77*, 239-245.

[152] Brinkworth, R.I.; Fairlie, D.P.; *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol*, **1995,** *1253,* 5-8.
[153] Makhija, M.T.; Kulkarni, V.M.; *J. Chem. Inf. Comput. Sci*, **2001,** *41,* 1569-1577.
[154] Emadi, A.; Harwood, J.S.; Kohanim, S.; Stagliano, K.W.; *Org. Lett*, **2002,** *4,* 521-524.
[155] Min, B.S.; Miyashiro, H.; Hattori, M.; *Phytother. Res*, **2002,** *16,* 557-562.
[156] Ilina, T.V.; Semenova, E.A.; Pronyaeva, T.R.; Pokrovsakii, A.G.; Nechepurenko, I.V.; Shults, E.E.; Andreeva, O.I.; Kochetkov, S.N.; Tolsrikov, G.A.; *Dokl. Biochem. Biophys*, **2002,** *382,* 56-59.
[157] Saller, R.; Buechi, S.; Megrat, R.; Schmidhauser, C.; *Forsch. Komplementarmed*, **2001,** *8,* 373-382.
[158] Hsiang, C.Y.; Hsieh, C.L.; Wu, S.L.; Lai, I.L.; Ho, T.Y.; *Am. J. Chin. Med*, **2001,** *29,* 459-467.
[159] Sendl, A.; Chen, J.L.; Jolad, S.D.; Stoddart, C.; Rozhon, E.; Kernan, M.; Nonakorn, W.; Balick, M.; *J. Nat. Prod*, **1996,** *59,* 808-811.
[160] Zhijie, W.; Pei, D.; Xuewen, F.; *Acta Acad. Med. Hubei*, **2000,** *21,* 180-183.
[161] Matsumoto, M.; Misawa, S.; Chiba, N.; Takaku, H.; Hayashi, H.; *Biol. Pharm. Bull*, **2001,** *24,* 236-241.
[162] Ertl, P.; Cooper, D.; Allen, G.; Slater, M.J.; *Bioorg. Med. Chem. Lett*, **1999,** *9,* 2863-2866.
[163] Fujiwara, A.; Mori, T.; Iida, A.; Ueda, S.; Hano, Y.; Nomura, T.; Tokuda, H.; Nishino, H.; *J. Nat. Prod*, **1998,** *61,* 629-632.
[164] Koyama, J.; Morita, I.; Tagahara, K.; Ogata, M.; Mukainaka, T.; Tokuda, H.; Nishino, H.; *Cancer Lett*, **2001,** *170,* 15-18.
[165] Koyama, J.; Morita, I.; Tagahara, K.; Osakai, T.; Hotta, H.; Yang, M.X.; Mukainaka, T.; Nishino, H.; Tokuda, H.; *Chem. Pharm. Bull*, **2001,** *49,* 1214-1216.
[166] Semple, S.J.; Pyke, S.M.; Reynolds, G.D.; Flower, R.L.P.; *Antiviral Res*, **2001,** *49,* 169-178.
[167] Ioset, J.R.; Marston, A.; Gupta, M.P.; Hostettmann, K.; *Phytochemistry*, **1998,** *47,* 729-734.

[168] Ioset, J.R.; Marston, A.; Gupta, M.P.; Hostettmann, K.; *Phytochemistry*, **2000,** *53,* 613-617.
[169] Haraguchi, H.; Yokoyama, K.; Oike, S.; Ito, M.; Nozaki, H.; *Arch. Microbiol*, **1997,** *167,* 6-10.
[170] Currelli, U.; Sollai, F.; Massa, L.; Comandini, O.; Rufo, A.; Sanjust, E.; Rinaldi, A.; Rinaldi, A. ; *J. Basic Microbiol*, **2001,** *41,* 253-259.
[171] Mahoney, N.; Molyneux, R.J.; Campbell, B.C.; *J. Agric. Food Chem*, **2000,** *48,* 4418-4421.
[172] Lee, S.E.; Campbell, B.C.; Molyneux, R.J.; Hasegawa, S.; Lee, H.S.; *J. Agric. Food Chem*, **2001,** *49,* 5171-5177.
[173] Agarwal, S.K.; Singh, S.S.; Verma, S.; Kumar, S.; *J. Ethnopharmacol*, **2000,** *72,* 43-46.
[174] Ali, M.I.A.; Shalaby, N.M.H.; Elgamal, M.H.A.; Mousa, A.S.M.; *Phytother. Res*, **1999,** *13,* 401-407.
[175] Jain, S,C.; Sharma, R.A.; Jain, R.; Mittal, C.; *Phytother. Res*, **1998,** *12,* 200-204.
[176] Khan, M.R.; Kihara, M.; Omoloso, A.D.; *Fitoterapia*, **2001,** *72,* 561-564.
[177] Hatano, T.; Vebayashi, H.; Ito, H.; Shiota, S.; Tsuchiya, T.; Yoshida, T.; *Chem. Pharm. Bull*, **1999,** *47,* 1121-1127.
[178] Liu, M.; Koya, S.; Furuta, H.; Matsuzaki, S.; *Dokkyo J. Med. Sci*, **1996,** *23,* 85-93.
[179] Manojlovic, N.T.; Solujic, S.; Sukdolak, S.; *Lichenologist*, **2002,** *34,* 83-85.
[180] Adeniyi, B.A.; Fong, H.H.S.; Pezzuto, J.M.; Luyengi, L.; Odelola, H.A.; *Phytother. Res*, **2000,** *14,* 112-117.
[181] Lall, N.; Meyer, J.J. ; *J. Ethnopharmacol*, **2001,** *78,* 213-216.
[182] Yang, X.; Summerhust, D.K.; Koval, S.F.; Ficker, C.; Smith, M.L.; Bernards, M.A.; *Phytother. Res*, **2001,** *15,* 676-680.
[183] Mehrabian, S.; Majd, A.; Majd, I.; *Aerobiologia*, **2000,** *16,* 455-458.
[184] Ali, N.A.A.; Julich, W.D.; Kusnick, C.; Lindequist, U.; *J. Ethnopharmacol*, **2001,** *74,* 173-179.
[185] Rothery, R.A.; Chatterjee, I.; Kiema, G.; McDermott, M.T.; Weiner, J.H.; *Biochem. J*, **1998,** *332,* 35-41.

[186] Bogdanov, P.; Gonzalez, M.; Sperandeo, N.R.; Fidelio, G.; Allesa, I.; *Biochem. Biophys. Res. Commun*, **1997,** *239,* 186-190.
[187] Bogdanov, P.M.; Ortiz, C.S.; Eraso, A.J.; Albesa, I.; *Med. Chem. Res,* **2001,** *10,* 577-586.
[188] Oliveira, C.G.T.; Miranda, F.F.; Ferreira, V.F.; Freitas, C.C.; Rabello, R.F.; Carballido, J.M.; Correo, L.C.D.; *J. Brazilian Chem. Soc,* **2001,** *12,* 339-345.
[189] Ruther, J.; Podsiadlowski, L.; Hilker, M.; *Chemoecology,* **2001,** *11,* 225-229.
[190] Danoun, S.; Baziard-Mousysset, G.; Stigliani, J.L.; Ane-Margail, M.; Payard, M.; Leger, J.M.; Canron, X.; Vial, H.; Bories, C.; *Heter. Comm,* **1999,** *5,* 343-348.
[191] Weiss, C.R.; Moideen, S.V.; Croft, S.L.; Houghton, P.J.; *J. Nat. Prod,* **2000,** *63,* 1306-1309.
[192] Kapadia, G.J.; Azuine, M.A.; Balasubramanian, V.; Sridhar, R.; *Pharmacol. Res,* **2001,** *43,* 363-367.
[193] Likhitwitayawuid, K.; Kaewamatawong, R.; Ruangrungsi, N.; Krungkrai, J.; *Planta Med,* **1998,** *64,* 237-241.
[194] Ittarat, I.; Asawamahasakda, W.; Barlett, M.S.; Smith, J.W.; Meshnick, S.R.; *Antimicrob. Agents Chemother,* **1995,** *39,* 325-328.
[195] Ball, M.D.; Barlett, M.S.; Show, S.; Smith, J.W.; Nasr, M.; Meshnick, S.R.; *Antimicrob. Agents Chemother,* **2001,** *45,* 1473-1479.
[196] Kayser, O.; Kiderlen, A.F.; Loatsch, H.; Croft, S.L.; *Acta Trop,* **2000,** *76,* 131-138.
[197] Kayser, O.; Kiderlen, A.F.; Loatsch, H.; Croft, S.L.; *Acta Trop,* **2000,** *77,* 307-314.
[198] Teixeira, M.J.; De Almeida, Y.M.; Viana, J.R.; Holanda, J.G.; Rodrigues, T.P.; Prata, J.R.C.; Coelho, I.V.B.; Rao, V.S.; Pompeu, M.M.L.; *Phytother. Res,* **2001,** *15,* 44-48.
[199] Sairafianpour, M.; Christensen, J.; Staerk, D.; Budnik, B.A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski, J.W.; *J. Nat. Prod,* **2001,** *64,* 1398-1403.
[200] Valderrama, J.A.; Benites, J.; Cortes, M.; Pessoa-Mahana, D.; Prima, E.; Fournet, A.; *Tetrahedron,* **2002,** *58,* 881-886.

[201] Moideen, S.V.K.; Houghton, P.J.; Rock, P.; Croft, S.L.; Aboagye-Nyame, F.; *Planta Med*, **1999,** *65,* 536-540.
[202] Morello, A.; Pavani, M.; Garbarino, J.A.; Chamy, M.C.; Frey, C.; Mancilla, J.; Guerrero, A.; Repetto, Y.; Ferreira, J.; *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol,* **1995,** *112,* 119-128.
[203] Goulart, M.O.F.; Zani, C.L.; Tonholo, J.; Freitas, L.R.; De Abreu, F.C.; Oliveira, A.B.; Raslan, D.S.; Starling, S.; Chiari, E.; *Bioorg. Med. Chem. Lett,* **1997,** *7,* 2043-2048.
[204] Salmon, C.L.; Lemaire, A.; De Freitas, S.; Deprez, B.; Sergheraert, C.; Davioud, C.E. ; *Bioorg. Med. Chem. Lett,* **2000,** *10,* 631-635.
[205] Salmon, C.L.; Buisine, E.; Yardley, V.; Kohler, S.; Debreu, M.A.; Landry, V.; Sergheraert, C.; Croft, S.L.; Krauth-Siegel, R.L.; *J. Med. Chem,* **2001,** *44,* 548-565.
[206] Grellier, P.; Sarlauskas, J.; Anusevicius, Z.; Maroziene, A.; Hovee-Levin, C.; Schrevel, J.; Cenas, N.; *Arch. Biochem. Biophys,* **2001,** *393,* 199-206.
[207] Zani, C.L.; Chiari, E.; Krettli, A.V.; Murta, S.M.F.; Cunningham, M.L.; Fairlamb, A.H.; Romanha, A,J.; *Bioorg. Med. Chem,* **1997,** *5,* 2185-2192.
[208] Valderrama, J.A.; Pessoa, M.D.; Tapia, R.A.; De Arias, A.R.; Nakayama, H.; Torres, S.; Miret, J.; Ferreira, M.E.; *Tetrahedron,* **2001,** *57,* 8653-8658.
[209] De Moura, K.C.G.; Emery, F.S.; Neves, P.C.; Pinto, M.D.F.R.; Dantos, A.P.; Salomao, K.; De Castro, S.L.; Pinto, A.V.; *J. Brazilian Chem. Soc,* **2001,** *12,* 325-338.
[210] Pinto, C.N.; Dantas, A.P.; De Moura, K.C.G.; Emery, F.S.; Polequevitch, P.F.; Pinto, M.D.; De Castro, S.L.; Pinto, A.V.; *Arzneimittelforsch,* **2000,** *50,* 1120-1128.
[211] Pinto, A.V.; Pinto, C.N.; Pinto, M.C.; Rita, R.S.; Pezzella, C.A.; De Castro, S.L.; *Arzneimittelforsch,* **1997,** *47,* 74-79.
[212] Camacho, M.R.; Kirby, G.C.; Warhust, D.C.; Croft, S.L.; Phillipson, J.D.; *Planta Med,* **2000,** *66,* 478-480.
[213] Khan, A.A.; Nasi, M.; Araujo, F.G.; *Antimicrob. Agents Chemother,* **1998,** *42,* 2284-2289.

[214] Khambay, B.P.S.; Batty, D.; Cahill, M.; Denholm, I.; Mead-Briggs, M.; Vinall, S.; Niemeyer, H.M.; Simmonds, M.S.; *J. Agric. Food Chem*, **1999,** *47,* 770-775.

[215] Khambay, B.P.S.; Jewers, P.; *Crop Proct*, **2000,** *19,* 597-601.

[216] Khambay, B.P.S.; Batty, D.; Beddie, D.G.; Denholm, I.; Cahill, M.R.; *Pestic. Sci*, **1997,** *50,* 291-296.

[217] Dos Santos, A.F.; Ferraz, P.A.L.; Pinto, A.V.; Pinto, M.C.F.R.; Goulart, M.D.F.; Sant'Ana, A.E.G.; *Int. J. Parasitol*, **2000,** *30,* 1199-1202.

[218] Dos Santos, A.F.; Ferraz, P.A.L.; De Abreu, F.C.; Chiari, E.; Goulart, M.D.F.; Sant'Ana, A.E.G.; *Planta Med*, **2001,** *67,* 92-93.

[219] Jewess, P.J.; Higgins, J.; Berry, K.J.; Moss, S.R.; Boogaard, A.B.; Khambay, B.P.S.; *Pest Manag. Sci*, **2002,** *58,* 234-242.

[220] Weissenberg, M.; Meisner, J.; Klein, M.; Schaeffer, I.; Eliyahu, M.; Schmutterer, H.; Ascher, K.R.S.; *J. Chem. Ecol*, **1997,** *23,* 3-18.

[221] Magdum, S.; Banerjee, S.; Kalena, G.P.; Banerji, A.; *J. Appl. Entomol*, **2001,** *125,* 589-596.

[222] Josephrajkumar, A.; Subrahmauyam, B.; Srinivason, V.; *Eur. J. Entomol*, **1999,** *96,* 347-353.

[223] Liu, S.Y.; Sporer, F.; Wink, M.; Jourdane, J.; Henning, R.; Li, Y.L.; Ruppel, A.; *Trop. Med. Int. Health*, **1997,** *2,* 179-188.

[224] Vandenbogaerde, A.; Zanoli, P.; Puia, G.; Truzzi, C.; Kamuhabwa, A.; De Witte, P.; Merlevede, W.; Baraldi, M.; *Pharmacol. Biochem. Behar*, **2000,** *65,* 627-633.

[225] Kalb, R.; Trautmann-Sponsel, R.D.; Niesel, M.; *Pharmacopsychiatry*, **2001,** *34,* 96-103.

[226] Franklin, M.; Cowen, P.J.; *Pharmacopsychiatry*, **2001,** *34,* S29-S37.

[227] Issopoulos, P.B. ; *Rev. Clin. Pharmacol. Pharmacokinet. Int. End*, **2000,** *14,* 32-42.

[228] Poutarad, A.; Di Gregorio, F.; Tin, V.C.F.; Girardin, P.; *Planta Med*, **2001,** *67,* 254-259.

[229] Briskin, D.P.; Gowienowski, M.C.; *Biochemistry*, **2001,** *39,* 1075-1081.

[230] Walker, L.; Sirvent, T.; Gibson, D.; Vance, N.; *Can. J. Bot*, **2001,** *79,* 1248-1255.

[231] Butterweck, V.; Wall, A.; Lieflander-Wulf, U.; Winterhoft, H.; Nahrstedt, A.; *Pharmacopsychiatry*, **1997,** *30,* 117-124.
[232] Butterweck, V.; Petereit, F.; Winterhoft, H.; Nahrstedt, A.; *Planta Med*, **1998,** *64,* 291-294.
[233] Misane, I.; Ogren, S.O.; *Pharmacopsychiatry*, **2001,** *34,* S89-S97.
[234] Wonnemann, M.; Singer, A.; Siebert, B.; Muller, W.E.; *Pharmacopsychiatry*, **2001,** *34,* S148-S151.
[235] Cott, J.M.; *Pharmacopsychiatry*, **1997,** *30,* 108-112.
[236] Raffa, R.B.; *Life Sci*, **1998,** *62,* PL265-PL270.
[237] Panocka, I.; Perfumi, M.; Angeletti, S.; Ciccocioppo, R.; Massi, M. ; *Pharmacol. Biochem. Behar*, **2000,** *66,* 105-111.
[238] Yu, P.H. ; *Pharmacopsychiatry*, **2000,** *33,* 60-65.
[239] Fornal, C.A.; Metzler, C.W.; Miressu, C.; Stein, C.K.; Jacobs, B.L. ; *Neuropsychopharmacology*, **2001,** *25,* 858-870.
[240] Kientsch, U.; Burgi, S.; Ruedeberg, C.; Probst, S.; Honegger, U.E.; *Pharmacopsychiatry*, **2001,** *34,* S56-S60.
[241] Simmen, U.; Higelin, J.; Berger-Buter, K.; Schaffner, W.; Lundstrom, K.; *Pharmacopsychiatry*, **2001,** *34,* S137-S142.
[242] Butterweck, V.; Bockers, T.; Korte, B.; Wittkowski, W.; Winterhoff, H.; *Brain Res*, **2002,** *930,* 21-29.
[243] Butterweck, V.; Winterhoff, H.; Herkenham, M.; *Mol. Phys*, **2001,** *6,* 547-564.
[244] Nahrstedt, A.; Butterweck, V.; *Pharmacopsychiatry*, **1997,** *30,* 129-134.
[245] Butterweck, V.; Jurgenliemk, G.; Nahrstedt, A.; Winterhoff, H.; *Planta Med*, **2000,** *66,* 3-6.
[246] Kulevanova, S.; Stefova, M.; Stafilov, T.; *Acta Pharm. Zagreb*, **2000,** *50,* 29-37.
[247] Muruganandam, A.V.; Ghosal, S.; Bhattacharya, S.K.; *Biogenc. Amin*, **2000,** *15,* 553-567.
[248] Wilhelm, K.P.; Biel, S.; Siegers, C.P.; *Phytomedicine,* **2001,** *8,* 306-309.
[249] Koch, E.; Chatterjee, S.S.; *Pharmacopsychiatry*, **2001,** *34,* S70-S73.
[250] Chatterjee, S.S.; Biber, A.; Weibezahn, C.; *Pharmacopsychiatry*, **2001,** *34,* S11-S19.

[251] Muller, W.E.; Singer, A.; Wonnemann, M.; *Pharmacopsychiatry*, **2001,** *34,* S98-S102.
[252] Philippu, A.; *Pharmacopsychiatry*, **2001,** *34,* S111-S115.
[253] Eckert, G.P.; Mueller, W.E.; *Pharmacopsychiatry*, **2001,** *34,* S22-S25.
[254] Klusa, V.; Germane, S.; Noldner, M.; Chatterjee, S.S.; *Pharmacopsychiatry*, **2001,** *34,* S61-S69.
[255] Barone, G.W.; Gurley, B.J.; Ketel, B.L.; Lightfoot, M.L.; Abul-Ezz, S.R.; *Ann. Pharmacother*, **2000,** *34,* 1013-1016.
[256] Moore, L.B.; Goodwin, B.; Jones, S.A.; Wisely, G.B.; Serabjit-Singh, C.J.; Willson, T.M.; Collins, J.L.; Kliewer, S.A.; *Proc. Natl. Acad. Sci. USA*, **2000,** *97,* 7500-7502.
[257] Durr, D.; Stieger, B.; Kullak-Ublick, G.A.; Rentsch, K.M.; Steinert, H.C.; Meier, P.J.; Fattinger, K.; *Clin. Pharm. Ther*, **2000,** *68,* 598-604.
[258] Moschella, C.; Jaber, B.L.; *Am. J. Kidney Dis*, **2001,** *38,* 1105-1107.
[259] Obach, R.S.; *J. Pharm. Exp. Ther*, **2000,** *294,* 88-95.
[260] Roby, C.A.; Anderson, G.D.; Kantor, E.; Dryer, D.A.; Burstein, A.H.; *Clin. Pharm. Ther*, **2000,** *67,* 451-457.
[261] Wang, Z.Q.; Gorski, C.; Hamman, M.A.; Huang, S.M.; Lesko, L.J.; Hall, S.D.; *Clin. Pharm. Ther*, **2001,** *70,* 317-326.
[262] Peebles, K.A.; Baker, R.K.; Kurz, E.U.; Scheneider, B.J.; Woll, D.J.; *Biochem. Pharmacol*, **2001,** *62,* 1059-1070.
[263] Renuka, R.; Rajasekeran, S.; Radhakrishnan, G.; *Appl. Biochem. Biotechnol*, **2001,** *96,* 83-92.
[264] Miles, D.H.; Payne, M.; *Tetrahedron*, **2001,** *57,* 5769-5772.
[265] Angelini, L.G. ; Pistelli, L. ; Belloni, P. ; Bertoli, A. ; Panconesi, S. ; *Ind. Crop. Prod*, **1997,** *6,* 303-311.
[266] Ali, R.; Sayeed, S.A.; *Antinut. Phytochem. Food,* **1997,** *662,* 223-244.
[267] Taylor, S.L.; Hefle, S.L.; *Allergy*, **2001,** *56,* 64-69.
[268] Acero, S.; Tabar, A.I.; Alvarez, M.J.; Garcia, B.E.; Olaguibel, J.M.; Moneo, I.; *Allergy*, **1998,** *53,* 897-901.
[269] Lizaso, M.T.; Moneo, I.; Garcia, B.E.; Acero, S.; Quirce, S.; Tabar, A.I.; *Ann. Allergy Asthma Immunol*, **2000,** *84,* 549-552.
[270] Reus, K.E.; Houben, G.F.; Stam, M.; Dubois, A.E.; *Ued. Tijdschrift. Geneeskd*, **2000,** *144,* 1836-1839.

[271] Chung, K.; Baker, J.R.; Baldwin, J.L.; Chou, A.; *Allergy*, **2001,** *56,* 73-77.

[272] Beaudouin, E.; Kanny, G.; Lambert, H.; Fremont, S.; Moneret-Vautrin, D.A.; *Ann. Allergy Asthma Immunol,* **1995,** *74,* 427-430.

[273] Wuthrich, B.; Kagi, M.K.; Stucker, W.; *Allergy*, **1997,** *52,* 1133-1137.

[274] Dicello, M.C.; Myc, A.; Baker, J.R.; Baldwin, J.L.; *Allergy Asthma Proc*, **1999,** *20,* 377-382.

[275] Steurich, F.; Feyerabend, R.; *Allergologie*, **2001,** *24,* 66-72.

[276] Kuramoto, Y.; Yamada, K.; Tsuruta, O.; Sugano, M:, *Biosci. Biotechnol. Biochem*, **1996,** *60,* 1712-1713.

[277] Baldwin, J.L.; Chou, A.H.; Solomon, W.R.; *Ann. Allergy Asthma Immunol,* **1997,** *79,* 415-419.

[278] Lisi, P.; Hansel, K. ; *Contact Dermatitis*, **1998,** *39,* 304-306.

[279] Bonamonte, D.; Foti, C.; Angelini, G. ; *Contact Dermatitis*, **2001,** *44,* 101-102.

[280] Estlander, T.; Jolanki, R.; Alanko, K.; Kanerva, L.; *Contact Dermatitis*, **2001,** *44,* 213-217.

[281] Robotham, J.M.; Teuber, S.S.; Sathe, S.K.; Roux, K.H.; *J. Allergy Clin. Immunol*, **2002,** *109,* 143-149.

[282] Majoie, I.M.; Bruynzeel, D.P.; *Am. J. Contact Dermat*, **1996,** *7,* 38-40.

[283] Lestringat, G.G.; Bener, A.; Frossard, P.M.; *Br. J. Dermatol*, **1999,** *141,* 598-600.

[284] Jappe, U.; Hausen-Bjoern, M.; Petzoldt, D.; *Contact Dermatitis*, **2001,** *45,* 249-250.

[285] Munday, R.; Smith, B.L.; Munday, C.M.; *Chem. Biol. Interact*, **1995,** *98,* 185-192.

[286] Kawazoe, S.; Hojo, Y.; Mizutani, T.; *J. Health Sci*, **2000,** *46,* 56-58.

[287] Munday, R.; Smith, B.L.; Munday, C.M.; *Chem. Biol. Interact*, **2001,** *134,* 87-100.

[288] Satoh, K.; Nagai, F.; Seto, T.; Yamauchi, H.; *Yakugaku Zasshi*, **2001,** *121,* 173-178.

[289] Gou, K.; Sen, L.; Lou, W.; *Chin. Pharm. J*, **1997,** *32,* 278-280.

[290] Wang, H.H.; Chung, J.G.; Ho, C.C.; Wu, L.T.; Chang, S.H.; *Planta Med*, **1998,** *64,* 176-178.

[291] Chung, J.G.; Tsou, M.F.; Wang, H.M.; Lo, H.M.; Hsieh, S.E.; Yen, Y.S.; Wu, L.T.; Chang, S.H.; Ho, C.C.; Hung, C.F.; *J. Appl. Toxicol*, **1998,** *18,* 117-123.

[292] Tamura, T.; Yokoyama, T.; Ohmori, K.; *Pharmacology*, **2001,** *63,* 228-233.

[293] Taniguchi, M.; Nagai, K.; Watanabe, M.; Nimura, N.; Suzuki, K.; Tanaka, A.; *J. Antibiot*, **2002,** *55,* 30-35.

[294] Arosio, B.; Gagliano, N.; Fusaro, L.M.P.; Parmeggiani, L.; Tagliabue, J.; Galetti, P.; De Castri, D.; Mocheni, C.; Annoni, G.; *Pharmacol. Toxicol*, **2000,** *87,* 229-233.

[295] Zhan, Y.; Li, D.; Wei, H.; Wang, Z.; Huang, X.; Xu, Q.; Lu, H.; *Chin. Med. J*, **2000,** *113,* 599-601.

[296] Goel, V.; Cheema, S.K.; Agellon, L.B.; Ooraikul, B.; Basu, T.K.; *Br. J. Nutr*, **1999,** *81,* 65-71.

[297] Oleynek, J.J.; Barrow, C.J.; Burns, M.P.; Sedlock, D.M.; Murphy, D.J.; Kaplita, P.V.; Sun, H.H.; Cooper, R.; Gillum, A.M.; Chadwick, C.C.; *Drug Dev. Res*, **1995,** *36,* 35-42.

[298] Ferreira, M.A.; Nunes, O.D.; Fujimura, A.H.; Pessoa, O.D.; Lemos, T.L.; Viana, G.S.; *Res. Commun. Mol. Pathol. Pharmacol*, **1999,** *106,* 97-107.

[299] Zhang, Y.H.; Chung, K.H.; Ryu, C.K.; Ko, M.H.; Lee, M.K.; Yun, Y.P.; *Biol. Pharm. Bull*, **2001,** *24,* 618-622.

[300] Suzuki, K.; Tatsuoka, T.; Ishihara, T.; Ogino, R.; Miyazaki, T.; Satoh, F.; Miyano, S.; Sumoto, K.; *Chem. Pharm. Bull*, **1997,** *45,* 668-674.

[301] Kim, S.R.; Lee, J.Y.; Lee, M.Y.; Chung, S.M.; Bae, O.N.; Chung, J.H.; *Toxicol. Sci*, **2001,** *62,* 176-182.

[302] Yim, T.K.; Wu, W.K.; Mak, D.H.; Ko, K.M.; *Planta Med*, **1998,** *64,* 607-611.

[303] Sato, M.; Moulik, G.; Bagchi, D.; Das, D.K.; *Free Radic. Res*, **2000,** *32,* 135-144.

[304] Neuhauscarlisle, K.; Vierling, W.; Wagner, H.; *Phytomedicine*, **1997,** *4,* 67-71.

[305] Saleem, R.; Faizi, S.; Siddiqui, B.S.; Ahmed, M.; Hussain, S.A.; Qazi, A.; Dar, A.; Ahmad, S.I.; Qazi, M.H.; Akhtar, S.; Hasnain, S.N.; *Planta Med*, **2001,** *67,* 757-760.

[306] Xu, C.; Yin, C.; Wang, S.; *Chin. Med. J*, **2001,** *114,* 571-576.
[307] Tanaka, J. ; *Nippon Rinsho*, **2001,** *59,* 2239-2244.
[308] Luo, J.; Cheung, J.; Yevich, E.M.; Clark, J.P.; Tsai, J.; Lapresca, P.; Ubillas, R.P. ; Fort, D.M. ; Carlson, T.J. ; Hector, R.F. ; King, S.R. ; Mendez, C.D. ; Jolad, S.D. ; Reaven, G.M. ; *J. Pharm. Exp. Ther*, **1999,** *288,* 529-534.
[309] Fort, D.; Ubillas, R.P.; Mendez, C.D.; Jolad, S.D.; Inman, W.D.; Carney, J.R.; Chen, J.L.; Ianiro, T.T.; Harbun, C.; Bruening, R.L.; Luo, J.; Reed, M.J.; Iwu, M.; Carlson, T.J.; King, S.R.; Bieser, D.E.; Cooper, R.; *J. Org. Chem*, **2000,** *65,* 6534-6539.
[310] Zhu, J.; Liu, Z.; Li, Y.; *Zhonghua Nei Ke Za Zhi*, **2001,** *40,* 537-542.
[311] Kim, D.M.; Park, E.K.; Bae, E.A.; Han, M.J.; *Biol. Pharm. Bull*, **2000,** *23,* 830-833.
[312] Cheng, H.H.; Kuo, S.C.; Lin, W.C.; *Res. Commun. Mol. Pathol. Pharmacol*, **1999,** *105,* 97-103.
[313] Oku, H.; Ishiguro, K.; *Phytother. Res*, **2001,** *15,* 506-510.
[314] Oku, H.; Kato, T.; Ishiguro, K.; *Biol. Pharm. Bull*, **2002,** *25,* 137-139.
[315] Yagi, A.; Hiue, N.; Asai, M.; Nakazawa, M.; Tateyama, Y.; Okamura, N.; Fujioka, T.; Mihashi, K.; Shimumura, K.; *Phytochemistry*, **1998,** *47,* 1267-1270.
[316] Sato, T.; Yamauchi, H.; Fukui, H.; *Kagawa Daigaku Nogakubu Gakujutsu Hokoku*, **1999,** *51,* 25-30.
[317] Arshi, A.; Abdin, M.Z.; Iqbal, M.; *Biologia Plantarum*, **2002,** *45,* 295-298.
[318] Rady, M.R.; Nazif, N.M.; *Fitoterapia*, **1997,** *68,* 349-354.
[319] Nazif, N.M.; Rady, M.R.; Seif El Nasr, M.M.; *Fitoterapia*, **2000,** *71,* 34-40.
[320] Guo, H.Z.; Chang, Z.Z.; Yang, R.J.; Guo, D.; Zheng, J.H.; *Phytochemistry*, **1998,** *49,* 1623-1625.
[321] Chang, Z.; Guo, D.A.; Shen, X.; Wang, S.; Zheng, J.; *Yaoxue Xuebao*, **1998,** *33,* 869-872.
[322] Tomova, L.; Dusek, J.; *Ceska. Slov. Farm*, **2000,** *49,* 78-81.
[323] Kasparova, M.; Siatka, T.; *Ceska. Slov. Farm*, **1999,** *48,* 256-261.
[324] Kasparova, M.; Dusek, J.; *Ceska. Slov. Farm*, **1999,** *48,* 132-135.

[325] Sepehr, M.F.; Ghorbanli, M.; *Plant Cell Tissue Organ Cult*, **2002,** *68*, 171-175.
[326] Kovacevic, N.; Subotic, A.; Budinir, S.; Grubisin, D.; *Pharm. Biol*, **2000,** *38*, 321-325.
[327] Han, Y.S.; Van der Heijden, R.; Veerpoorte, R.; *Plant Cell Tissue Organ Cult*, **2001,** *67*, 201-220.
[328] Shim, J.J.; Shim, J.H.; Pai, T.; Chung, I.S.; Lee, H.J.; *Biotechnol. Tech*, **1999,** *13*, 249-252.
[329] Stara, D.; Suchy, V.; Blanarik, P.; *Ceska. Slov. Farm*, **1995,** *44*, 167-169.
[330] Boka, K.; Jakas, J.; Kiraly, I.; *Biologia Plantarum*, **2002,** *45*, 281-290.
[331] Mantrova, O.V.; Dunaeva, M.V.; Kuzovkina, I.N.; Schneider, B.; Muller-Uri, F.; *Russ. J. Plant Physiol*, **1999,** *46*, 248-251.
[332] Lodhi, A.H.; Charlwood, B.V.; *Plant Cell Tissue Organ Cult*, **1996,** *46*, 103-108.
[333] Abdullah, M.A.; Ali, A.M.; Marziah, M.; Lajis, N.H.; Ariff, A.B.; *Plant Cell Tissue Organ Cult*, **1998,** *54*, 173-182.
[334] Hagendoorn, M.J.; Jamar, D.C.; Van der Plas, L.H.; *Methods Mol. Biol*, **1999,** *111*, 383-391.
[335] Abdullah, M.A.; Ariff, A.B.; Marziah, M.; Ali, A.M.; Lajis, N.H.; *J. Agric. Food Chem*, **2000,** *48*, 4432-4438.
[336] Abdullah, M.A.; Ariff, A.B.; Marziah, M.; Ali, A.M.; Lajis, N.H.; *Plant Cell Tissue Organ Cult*, **2000,** *60*, 205-212.
[337] Hagendoorn, M.J.M.; Jamar, D.C.L.; Meykamp, B.; Van der Plas, L.H.W.; *J. Plant Physiol*, **1997,** *150*, 325-330.
[338] Kitajima, M.; Fischer, V.; Nakamura, M.; Ohsawa, M.; Neno, M.; Takayama, H.; Unger, M.; Stockigt, J.; Aimi, N.; *Phytochemistry*, **1998,** *48*, 107-111.
[339] Dornenburg, H.; Knorr, D.; *J. Biotechnol*, **1996,** *50*, 55-62.
[340] Baker, R.A.; Tatum, J.H.; *J. Ferment. Bioeng*, **1998,** *85*, 359-361.
[341] Arrebola, M.L.; Ringbom, T.; Verpoorte, R.; *Phytochemistry*, **1999,** *52*, 1283-1286.
[342] Schripsema, J.; Ramos, V.A.; Verpoorte, R.; *Phytochemistry*, **1999,** *51*, 55-60.

[343] Han, Y.S.; Van der Heijden, R.; Lefeber, A.W.M.; Erkelens, C.; Veerpoorte, R.; *Phytochemistry*, **2002,** *59,* 45-55.
[344] Ramos, A.C.; Van der Heijden, R.; Verpoorte, R.; *Planta,* **1997,** *203,* 155-161.
[345] Kim, C.Y.; Im, H.W.; Kim, H.K.; Huch, H.; *Appl. Microbiol. Biotechnol,* **2001,** *56,* 239-242.
[346] Panichayupakanont, P.; *Pharm. Biol,* **2001,** *39,* 293-296.
[347] Budzianowski, J.; *Planta Med,* **2000,** *66,* 667-669.
[348] Hook, I.; Walsh, J.; Kavanagh, P.; Reininger, R.; *Pharm. Pharmacol. Lett,* **1997,** *7,* 93-95.
[349] Bringmann, G.; Rischer, H.; Wohlfarth, M.; Schlauer, I.; Assi, L.A.; *Phytochemistry,* **2000,** *53,* 339-343.
[350] Hook, I.L.I.; *Plant Cell Tissue Organ Cult,* **2001,** *67,* 281-285.
[351] Budzianowski, J.; *Phytochemistry,* **1997,** *44,* 75-77.
[352] Bulgakov, U.P.; Kozyrenko, M.M.; Fedoreyev, S.A.; Mischenko, N.P.; Denisenko, V.A.; Zvereva, L.V.; Pokushalova, T.V.; Zhuravlev, Y.N.; *Fitoterapia,* **2001,** *72,* 394-401.
[353] Bais, H.P.; Walker, T.S.; McGrew, J.J.; Vivanco, J.M.; *In vitro Cell. Dev. Biol. Plant,* **2002,** *38,* 58-65.
[354] Kirakosyan, A.; Hayashi, H.; Inoue, K.; Charchoglyan, A.; Vardapetyan, H.; *Phytochemistry,* **2000,** *53,* 345-348.
[355] Kirakosyan, A.; Vardapetyan, R.R.; Charchoglyan, A.G.; Yamamoto, H.; Hayashi, H.; Inoue, K.; *Russ. J. Plant Physiol,* **2001,** *48,* 816-819.

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# SEROTONIN (5-HYDROXYTRYPTAMINE) AND THE ETIOLOGY OF AUTISM

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**ABSTRACT:** 5-Hydroxytryptamine (5-HT), also known as serotonin, is a small molecule synthesized from tryptophan. The serotonergic cells project their axons to a large proportion of neurons, which explains the involvement of 5-HT in many brain physiological mechanisms, behaviors and disease processes. Autism is classified as a pervasive developmental disorder characterized by impairments in three human behavioral domains: a) communication, both verbal and nonverbal, b) socialization (interaction with others) and c) restricted stereotyped behaviors and interests. Symptoms are detectable before the age of three. The etiology of autism is unknown, but it has a genetic basis. Several lines of evidence suggest that 5-HT may be involved in the pathogenesis of autism. For instance, an increase in whole blood and platelet serotonin levels has been reported in about one third of autistic subjects. Furthermore, some autistic patients respond favorably to serotonin re-uptake inhibitors. As serotonergic innervations play an important role in brain development, various neuroanatomical abnormalities associated with autism may be secondary to early dysfunction of this system. Finally, some genetic studies have yielded modest evidence for linkage and association of autism to the serotonin transporter and receptor genes. The possible involvement of genes related to synthesis, release, transport, degradation and signal reception of 5-HT in autism is discussed.

## INTRODUCTION

Serotonin is an indolamine related to the amino acid tryptophan. In evolutionary terms it is considered to be one of the oldest neurotransmitters, and several lines of evidence show that it is involved in more physiological mechanisms, behaviors and brain processes than any other neurotransmitter. In addition to its role as a neurotransmitter, 5-HT has morphogenic properties and can alter the cytoskeleton of cells. It also acts as a regulator of cell proliferation, migration, apoptosis and maturation in a variety of tissues and cell types, including lung, kidney, endothelial cells, mast cells, astrocytes and neurons [1].

Serotonergic neurons are usually large, multipolar, clustered and located mainly on the midline of the brain stem forming the raphe nuclei. These nuclei contribute to the regulation of attention and other complex cognitive functions. In humans, serotonergic neurons start to be present between the fifth and the sixth week of gestation. They are among the first neurons to differentiate in the brain, playing a key role in the regulation of neurogenesis [2]. Abnormalities of serotonergic neurotransmission may thus affect neuronal differentiation, proliferation and/or synaptogenesis during development, resulting in cognitive and behavioral dysfunction.

5-HT has been implicated in the etiology of numerous diseases, such as migraine, hypertension, irritable bowel syndrome and many mental disorders, including depression, anxiety, social phobia, schizophrenia, obsessive-compulsive and panic disorders [3]. It also seems to be involved in human mood and social interaction [4]. Therefore, it is not a surprise that the vast distribution of serotonergic fibers in the brain interact with a large variety of cell types, including neurons, glial, ependymal and endothelial cells, through at least 14 distinct receptor proteins [5-7].

## Autism and serotonin: the neurochemical connection

Autism is a pervasive developmental disorder manifested by impairments in reciprocal social interaction, limited or absent language, and stereotyped and restricted patterns of activities and interests [8, 9]. Elevated levels of whole blood or platelet 5-HT have been consistently detected in one third of autistic subjects [10, 11] and in their first-degree relatives [12-14]. This well replicated hyperserotonemia has focused attention on the possible involvement of this neurotransmitter in the etiology of autism. Other observations also suggest that a dysregulation of serotonergic neurotransmission could be related to the pathogenesis of autism. For instance, a dietary reduction of tryptophan, the 5-HT precursor, in adult autistic patients results in an aggravation of behavioral symptoms such as whirling, flapping, pacing and self-hitting [15]. On the other hand, 5-HT re-uptake inhibitors, which block uptake of 5-HT into the presynaptic neuron, seem to alleviate some autistic symptoms such as ritualistic and aggressive behaviors [16, 17]. It has also been suggested that abnormalities of serotonergic transmission may predispose

genetically susceptible individuals to autism, because changes in levels of serotonin during fetal life, and/or at some time in the first two years after birth, are thought to be critical for appropriate brain neurodevelopment [18, 19].

## CHEMICAL STRUCTURE AND SYNTHESIS OF SEROTONIN

Serotonin is synthesized from tryptophan, which is the only amino acid containing an indole ring, composed of a benzene molecule and a secondary pentane ring with a central nitrogen. This indole ring has the property of light absorption. In plants, tryptophan is essential for the action of light receptor molecules; for instance, it is present in chlorophyll, which captures photons generating adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH) cofactor. The interaction between light and tryptophan is also important in mammalian systems, since the pineal hormone melatonin is synthesized from tryptophan through serotonin and is involved in the regulation of circadian rhythmicity [20, 21]. Abnormalities of circadian activity are thought to underlie a wide variety of psychiatric disorders, including seasonal affective disorder, bipolar disorder, unipolar depression, bulimia, anorexia, schizophrenia, panic disorder, and obsessive compulsive disorder [22].

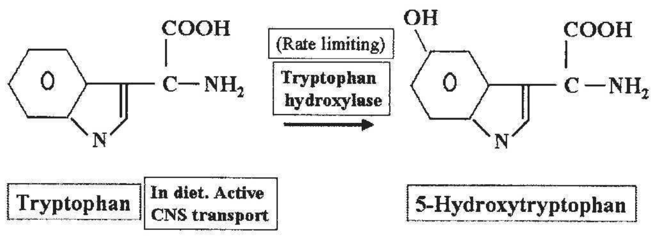


**Fig. (1).** Reaction carried out by the enzyme tryptophan hydroxylase. Tryptophan is supplied by dietary intake and transported actively to the central nervous system (CNS). Tryptophan hydroxylase is the key enzyme controlling the synthesis of serotonin and is predominantly present in neurons located in the raphe nuclei of the brain stem

The synthesis of serotonin from tryptophan is carried out in two steps controlled by two enzymes: tryptophan hydroxylase (TPH) and aromatic L-amino acid decarboxylase (AADC). The second enzyme, AADC, is also known as DOPA carboxylase or 5-hydroxytryptophan carboxylase when it acts specifically in 5-HT synthesis. In the first step, the TPH adds a hydroxyl chemical group (-OH) to tryptophan to make 5-hydroxytryptophan, Fig **(1)**. In the second step, AADC removes the carboxyl group (-COOH) from 5-hydroxy tryptophan to make serotonin, Fig **(2)**.

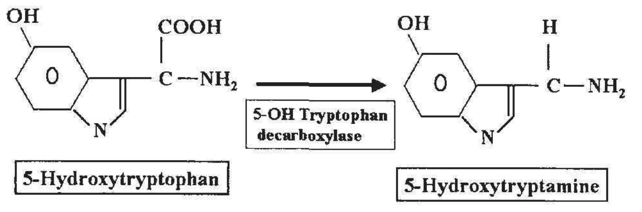


**Fig (2).** Aromatic L-amino acid decarboxylase also known as tryptophan decarboxylase, catalyses the synthesis of 5-hydroxytriptamine (serotonin) from 5-hydroxytryptophan. The reaction consists of a decarboxylation activity that is found in many human tissue cells

The rate limiting step in the production of serotonin is controlled via TPH, an enzyme that is present only in specialized neurons located chiefly in the raphe nuclei of the brain stem, although a number of neurons located in different parts of the central nervous system, such as the spinal cord, hippocampus and the cerebral cortex, also have TPH activity [23]. This activity is also detected in other specialized tissues unrelated to the nervous system, such as mast cells, mononuclear leukocytes, beta-cells of the islets of Langerhans, and intestinal and pancreatic enterochromaffin cells. There is a similarity between the mechanism of hydroxylation involving TPH and tyrosine hydroxylase, the key enzyme in the biosynthesis of catecholamines. The sequence of amino acids of both enzymes are also alike and both genes are close to one another in the genome (TPH: 11p15.3-p14, tyrosine hydroxylase: 11p15.5), which suggests that both enzymes may be derived from a genetic duplication of an ancestral gene.

The enzyme AADC is involved in different metabolic pathways synthesizing two important neurotransmitters: dopamine and serotonin [24]. AADC decarboxylates L-dihydroxy-phenylalanine to form dopamine and 5-hydroxytryptophan to produce serotonin. Tryptophan decarboxylase activity is detected in many brain neurons and non-nervous tissue cells.

## AUTISM: A COMPLEX DISORDER WITH MULTIPLE ETIOLOGIES

Autism, as we have stated in the introduction, is a neurodevelopmental disorder characterized by early onset of impairments in social interaction and communication, accompanied by restricted, repetitive and stereotyped patterns of behavior. Several theories on the etiology of autism, ranging from psychological to biological, have been proposed. The idea that genetic factors are strongly involved in this disorder has been generally accepted, and today autism is considered the most heritable complex mental disorder, a proposition that is supported by family, twin and molecular genetics studies.

Family studies provide a sibling recurrence risk of 2%-6% [25-28], which means that the siblings of autistic individuals are about 20-120 times more likely to develop autism that a person in the general population (population prevalence: 0.05 to 0.1%). Notably, there is a substantial fall off in risk of autism for second and third degree relatives of autistic probands [28], suggesting that multiple interacting genes may be required to produce the syndrome. Four twin studies show a consistently higher concordance rate in monozygotic (MZ) versus dizygotic (DZ) twins, further supporting a genetic component to autism [29-32]. In general, the studies demonstrate a MZ pair-wise concordance rate between 36%-91% with a DZ pair-wise concordance of 0 (this should be 2-6% as with regular sibs but 0 appears because the sample size was small). Finally, genome screening studies of autistic sibling pair samples have yielded suggestive linkage to multiple sites, but only some findings are consistent across studies. The regions where there seems to be the most consistent evidence for linkage are on chromosomes 7q, 2q and 16p [33].

Autistic symptoms develop prior to the age of 3 and persist throughout life. Approximately 75% of cases are mentally retarded and 25-35% of persons develop epilepsy [34]. The diagnostic criteria for autism are summarized in Table 1.

**Table 1.- Diagnostic criteria for Autistic Disorder (based on DSM-IV).**

| Domain | Symptom |
|---|---|
| Social interaction | a. Marked impairment in the use of multiple nonverbal behaviors to regulate social interaction (e.g. facial expression).<br>b. Failure to develop peer relationships.<br>c. Lack of spontaneous seeking to share enjoyment with others.<br>d. Lack of social or emotional reciprocity (e.g. preferring solitary activities). |
| Communication | a. Delay in, or total lack of the development of spoken language.<br>b. In individuals with adequate speech, marked impairments in initiating or sustaining a conversation with others.<br>c. Stereotyped and repetitive use of language or idiosyncratic language.<br>d. Lack of varied, spontaneous make-believe play or social imitative play. |
| Pattern of behavior | a. Preoccupation with stereotyped or restricted patterns of interests.<br>b. Inflexible adherence to specific, nonfunctional routines or rituals.<br>c. Stereotyped and repetitive motor mannerisms (e.g. hand flapping).<br>d. Persistent preoccupation with parts of objects. |

As can be deduced from Table 1, autism is a syndrome with a wide variety of behavioral symptoms that vary in severity. In the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) of the American Psychiatric Association [35], autism is considered to be on a continuum with other related disorders, called Pervasive Developmental Disorders (PDDs). These include five other official diagnoses, characterized by deficits in communication and social skills; however, differences in language development severity and age of onset demarcate each of the subtypes. The group of PDDs includes autistic disorder (AD, previously referred to as classic autism), Asperger's syndrome (AS), Rett's disorder (RTT), childhood disintegrative disorder (CDD), and pervasive developmental disorder-not otherwise specified (PDD-NOS). Table 2 summarizes some particular characteristics and criteria distinctive of the five kinds of PDDs.

Although autism and, in general, PDDs are very heterogeneous disorders, AD, AS and CDD might be considered analogous diseases with variable degrees of expression. CDD has behavioral symptoms remarkably similar to those of AD, except that it is characterized by a later age of onset. However, as it is very rare, persons with CDD have not

been included in genetic studies. AS is thought to be a less severe form of autism and is characterized by social/behavioral deficits without significant language or cognitive delays. Multiple studies convincingly demonstrate that autism and milder phenotypic variants appear to share the same genetic liability [27, 36, 37].

**Table 2.- Some diagnostic criteria distinctive for PDDs (based on DSM-IV).**

| Item | AD | AS | CDD | RTT | PDD-NOS[a] |
|---|---|---|---|---|---|
| Sex | Increased male to female ratio | Increased male to female ratio | Increased male to female ratio | Only in female | - |
| Age of onset | 0-3 years | Usually 0-3 years | 3-10 years | Usually 0-3 years | - |
| Onset: Speech delay | Yes | No | Yes | No | - |
| Onset: Cognitive delay | Yes | No | Yes | Yes | - |
| Onset: Motor delay | No usual | May be present | Yes | Yes | - |
| Mental Retardation | IQ could be from normal to less than 50 | No | Profound to severe | Profound to severe | - |

[a]Pervasive developmental disorders with impairment in reciprocal social interaction or verbal and nonverbal communication skills which do not satisfy the criteria of the other disorders considered inside the spectrum.

RTT, however, has a unique genetic etiology and can be separated from other PDDs which appear to share genes in common. Unlike the other PDDs that demonstrate an increased male to female sex ratio of about 4:1, RTT is a disorder affecting exclusively females and is characterized by normal development for the first 6–18 months of life, followed by developmental decay and loss of acquired skills. Unlike the other PDDs, the genetic basis of Rett syndrome is well established. The disorder is monogenic and the responsible gene lies on the X chromosome. RTT is caused by mutations in the *MECP2* gene, which encodes methyl CpG-binding protein 2. This protein regulates gene expression through its interaction with methylated DNA [38]. Occasionally, autistic children may carry a mutation in the *MECP2* and do not present the typical symptoms of Rett syndrome. In a study of 60 girls diagnosed with autism, without classic signs of Rett syndrome, two had mutations in the *MECP2* gene [33].

According to current epidemiological data from the M.I.N.D. (Medical Investigation of Neurodevelopmental Disorders) Institute in California, the prevalence and incidence of autism is definitely increasing, at least in the United States. Prior to the 1990s, the population prevalence was calculated to be 5 per 10,000 for autism and 20 per 10,000 for total PDDs [39]. Meanwhile, recent publications, have reported rates of about 20 per 10,000 for autistic disorder, and about 60 per 10,000 for total PDDs. These studies were conducted in 13 countries, including USA, UK, Japan, France, Sweden, Norway, Finland, Iceland, Denmark, Indonesia, Germany, Ireland and Canada. There was some suggestion previously that the increased prevalence of autism was due to improved diagnostic procedures; however, the California data from the year 2002 seem to disprove this hypothesis. Of note, it appears that there may be a downward trend in the rate of mental retardation in children diagnosed with autism, from 75% down to a current rate as low as 25% [39-41], although there is no current explanation for this finding.

Autistic features are also seen in patients with major medical illnesses or chromosomal defects [33], such as phenylketonuria, tuberous sclerosis, or fragile X syndrome, and in patients exposed to certain environmental factors in utero, including hypothyroidism, congenital cytomegalovirus or rubella infections and maternal thalidomide or alcohol consumption. Approximately 5% of individuals with autism have an underlying chromosomal anomaly, the most frequent of which involves duplication of the Prader-Willi/Angelman syndrome region of chromosome 15. Therefore, autism may be a component feature of several genetic and environmental factors. In spite of this etiological heterogeneity, a shared neurochemical pathway may still underlie autistic spectrum disorders, as different genes may affect different portions of a common pathway [42].

## AUTISM AND SEROTONIN: THE NEUROANATOMICAL CONNECTION.

The most essential stage in human brain development occurs during the prenatal period and the first four year of life. Just after birth, the intrinsic nature of the individual, including epigenetic and genetic information, combines with extrinsic environmental factors and act in concert to modify brain domains to cope with cognitive, social, linguistic and other

intellectual skills. The fact that dizygotic twins share the same environment in utero and yet one may develop normally, even though the other has autism, suggests a strong intrinsic or genetic component to the illness.

A variety of neuroanatomic differences have been observed in samples of autistic patients in comparison with controls. This topic has been reviewed in recent publications [43-45]. Magnetic resonance imaging (MRI) studies show that by 2-4 years of age, 90% of autistic children have an abnormal early brain overgrowth followed by abnormally slowed growth [45, 46]. The most common finding has been hyperplasia of cerebral gray matter and cerebral and cerebellar white matter in the early life of autistic patients [46].

The results presented in different reports point to particular brain structures that have been found to be anatomically affected in autistic persons. These are the cerebellum, the amygdala and the hippocampus [43, 47, 48].

Several studies have demonstrated the existence of a decrease in the number of Purkinje cells in the cerebellum of autistic individuals [49-51]. 5-HT might be related to this anatomical abnormality because a high level of mRNA expression of the 5-HT5A receptor has been found in normal Purkinje cells [52]. Furthermore, this receptor appears to be involved in brain development because 5-HT5A mRNA was detected at embryonic day 18 in the rat central nervous system and peaked by postnatal day 20 [53]. Knockout mice lacking 5-HT5A receptor activity exhibited phenotypic behavioral irregularities, including increased exploratory activity, with respect to controls [54]. In addition, Pierce and Courchesne [55] have reported that in a group of autistic children there is a positive correlation between a decrease in the number of Purkinje cells (cerebellar hypoplasia) and a reduction in the time they participate in active exploration.

Several lines of evidence also suggest that a deficit in the development of the amygdala, a collection of nuclei located in the medial temporal lobe, might be involved in autism. Both a reduction in the volume and activation of the amygdala during mental tasks has been observed in some autistic subjects (reviewed in [56]). The amygdala, develops 30-50 days after zygote formation, although some separate nuclei do not differentiate until birth. This development pattern suggests that there may be significant plasticity in response to environmental signals [57]. Interestingly, Hariri et al [58] have reported that a short allele of the

promoter region of the human serotonin transporter gene (*SLC6A4*, see section 6 of this review) is associated with greater neuronal activity in the amygdala in response to fearful stimuli and also with deficits in facial processing of emotions (a key trait in autism), compared with homozygosity of the long allele. Recently Weidenfeld et al [59] found that projections from the amygdala facilitate the function of dorsal raphe 5-HT neurons. Furthermore, it is known that 5-HT availability influences fear [60] as well as pathological anxiety [61], both behaviors dependant upon the amygdala [62].

Neuropathological studies in autistic brains have also demonstrated smaller neuronal size and increased cell packing density in the hippocampus, another limbic system structure. The morphology of hippocampal neurons in two cases of infantile autism was studied and investigators found smaller cells with reduced perikaryon area and dendritic branching in comparison with neurons of age-matched control children [63]. This result is consonant with MRI results evidencing a smaller area dentata of the hippocampus in autistic children from 2 to 4 years [64]. However, the density and distribution of some 5-HT receptors in the hippocampus were not statistically different between autistic children and controls [65].

## SEROTONIN AND BRAIN DEVELOPMENT.

As mentioned previously, serotonin has been shown to play a role in regulating brain growth [1]. Changes in serotonin synthesis, serotonergic innervation and serotonin receptor density may all influence brain development.

### Serotonin synthesis

The highest functional status of the serotonin system is reached very early in development. In the human brain, serotonergic neurons are present by 5 weeks of gestation [66], and increase quickly throughout the $10^{th}$ week [67-69]. The typical distribution of serotonergic neurons in the raphe nuclei can occur in the $15^{th}$ week [70]. After this stage, serotonin levels continue increasing, but more slowly, during the first 2 years of life and then decline to adult levels by 5 years of age [71, 72]. Using alpha(C-

11)methyl-L-tryptophan and positron emission tomography (PET), Chugani et al [73] have demonstrated global brain changes in the capacity to synthesize serotonin between autistic children and their non-autistic siblings and also epileptic children without autism. For non-autistic children, serotonin synthesis capacity is over 200% of adult values until the age of 5 years, and then later declines to adult value. On the other hand, in autistic children the serotonin synthesis increases gradually between the age of 2 and 11 years, reaching values one and a half times the adult value. Also, asymmetries of serotonin synthesis in the frontal cortex, thalamus, and dentate nucleus of the cerebellum in autistic children have been demonstrated by PET [74]. These data indicate that the human brain experiences a period of high serotonin synthesis during early development and childhood, and at these stages it is probable that the neurotransmitter influences neuronal outgrowth and synaptogenesis [75]. In autistic children, the normal developmental process could become affected by impaired brain regions originated by mutations affecting 5-HT synthesis, regulation or signal reception.

**Serotonergic innervations.**

The development of serotonergic innervation to the cerebral cortex has been characterized in neonatal rats by immunohistochemical techniques and autoradiographic imaging. These experiments demonstrate early serotonergic innervation that could be involved in a transient physiological role in sensory areas of the cortex or exert a trophic influence on the development of cortical circuitry and thalamocortical connections [76].

Experiments using both knock-out and pharmacological approaches have demonstrated that serotonin plays a role in modulating synaptogenesis in rodents. Monoamine oxidase (MAO) is an enzyme which degrades neurotransmitters such as noradrenaline, dopamine, and serotonin. There are two distinct forms of the enzyme, A and B, which are encoded by two separate genes, both with 15 exons and lying adjacent to each other on human Xp11.23-11.4 [77]. Both MAO A and B deaminate dopamine but have some different characteristics with respect to time of expression and other enzymatic activities: MAOA preferentially deaminates serotonin and noradrenaline and its maximal activity appears in the fetal brain. MAOB mainly deaminates neurotransmitters

phenylethylamine and benzylamine and its activity is higher in the adult brain. MAOA knockout pup mice exhibit up to a nine-fold increase in the concentration of brain serotonin. It seems that elevated amounts of this neurotransmitter are responsible for the increase in arborization of the axons, resulting in haziness of the boundaries of the cortical barrels of the mouse brain. These experiments suggest that serotonergic irregularities may be contribute to these cortical abnormalities, since early administration of parachlorophenylalanine, an inhibitor of serotonin synthesis, restored the formation of barrels, whereas inhibition of catecholamine synthesis did not [78]. It has also been demonstrated that depletion of serotonin delays the development and decreases the size of the barrel fields [79]. Therefore, a decreased or increased level of brain serotonin during the period of brain development results in a disruption of synaptogenesis in the sensory cortices.

The effect of serotonin on synaptogenesis is not limited to the sensory cortex, as it has also been reported that reduction of serotonin in the early postnatal period can result in changes in the morphology of hippocampal dentate granule cells, particularly at the level of the synapse, as reflected by the permanent reduction in synaptic spine density [80].

## Serotonin receptor density

It has been reported that serotonin can produce opposite and complementary action on neuronal functioning, maturation, proliferation and apoptosis, depending on the kind and the density of receptors synthesized by the cells [1]. There are at least fourteen different receptor subtypes described for 5-HT, 13 different heptahelical G-protein-coupled receptors and one ligand-gated ion channel (Table 3). The 5-HT receptor family has been, and is at present, subjected to intense research. Excellent and complete reviews of functional, molecular and pharmacological aspects of 5-HT receptors have been published [5-7].

The role of 5-HT during development seems essential since the neurotransmitter acts as a developmental signal in the early embryonic central nervous system [81]. In rats, mature serotonergic innervation occurs during the first three weeks of the embryonic stage [82], a process accompanied by a two-fold increase in serotonin [83]. Postnatal treatment with 5-HT1AR receptor antagonists decreased dendritic spine density in developing dentate gyrus cells [84]. Gross et al [85] have carried out

experiments in mice that strongly support the idea that normal anxiety-like behavior requires serotonin 5-HT1A receptors in the hippocampus and cortex during the early postnatal period. Activation of these receptors may be an essential part of the pathway that promotes changes in brain structure that are necessary for normal anxiety-like behavior during adult life.

In-situ hybridization experiments in the human brain have demonstrated specific mRNA expression of the 5-HT5A receptor in the cortex and in limbic structures such as the hippocampus and the ventrolateral region of the amygdala. These are areas involved in cognitive and emotional processes [86]. Also, 5-HT receptors (5HT5A and others) have been detected in Purkinje cells, the granular layer and dentate nucleus of the cerebellum [86], a structure already associated with autism by neuroanatomical and imaging studies. On the other hand, the 5HT5A receptor is poorly expressed or absent in areas involved in motor function, such as the basal ganglia and the substantia nigra, regions not affected in autistic children [87].

## CANDIDATE GENES FOR AUTISM INCLUDE THOSE INVOLVED IN THE SYNTHESIS, RELEASE, TRANSPORT, DEGRADATION AND RECEPTOR ACTIVITY OF SEROTONIN.

Genes acting on the central nervous system during fetal life and in the first two years after birth may all be considered candidate autism susceptibility genes. Between these are the genes involved in the synthesis, release, transport, degradation and signal receptors of neurotransmitters.

### Synthesis

We have mentioned in the second section of this review that tryptophan hydroxylase (TPH) is the rate limiting enzyme in the synthesis of 5-HT. The human TPH gene was localized to chromosome 11p14-p15.3. The enzyme is tetrameric [88]. A polymorphism within intron 7 has been detected and that may influence 5-HT metabolism in the brain [89]. Hong et al [90] described an association between the TPH gene polymorphism (*A218C*) and schizophrenia. At present there are no association studies of autism and this polymorphism. However, the fact that the ontogeny of

TPH mRNA and enzyme activity occurs primarily in the raphe region during the early postnatal period, from day 8 to 22 in rats [23], makes this gene and the genes controlling its activity attractive candidates for association studies.

The activity of the aromatic L-amino acid decarboxylase (AADC) is implicated in different metabolic pathways, including the synthesis of 5-HT by means of its tryptophan decarboxylase avtivity [24]. AADC is a homodimeric pyridoxal phosphate-dependent enzyme. The gene coding this enzyme is located in chromosome 7p11 and consist of 15 exons spanning more than 85 kb [91]. Chatelin et al [92] have studied the expression of the AACD human gene in transgenic mice carrying the promoter of the gene in a 3.6 kb region flanking the first exon. They showed that the gene is highly expressed on the substantia nigra, the ventral tegmental area and in the nuclei raphe dorsalis, medialis and pontis. Deficiency of AADC activity causes a rare disorder with multiple phenotypic characteristics, including eye movement abnormalities, abnormal sleeping, and feeding dificulties with esophagea reflux [93].

## Release

The gene *SLC18A2* is located on chromosome 10q25 and codes for the vesicular monoamine transporter type 2 (VMAT2) [94]. It loads 5-HT and other monoamines into presynaptic vesicles and regulates the supply of 5-HT available for release. It has been found that VMAT2 inhibitors abolished aggressive behavior of the *MAOA* gene in knockout mice [95]. No functional polymorphisms in this gene have been identified in humans and therefore studies of its influence on behavior have not been conducted.

## Transport

The cause or causes and the meaning of the elevation of platelet 5-HT observed in some autistic patients is unknown, but the serotonin transporter (5-HTT) is thought to be involved as it controls the rate of reuptake of serotonin from the synaptic cleft. The fact that selective serotonin reuptake inhibitors ameliorate obsessive compulsive symptoms, stereotypies, affective liability and social relatedness in some subjects with autism [16, 17], lends further support for this hypothesis. After

serotonin release in brain synapses, it is taken up by the 5-HTT into the presynaptic neuron, which thus terminates the synaptic actions of serotonin and recycles it into the neurotransmitter pool. One of the reasons why hyperserotonemia occurs in autism could be to enhance 5-HT uptake into platelets, since free 5-HT levels seem to be normal [96, 97]

The 5-HTT gene, named *SLC6A4* (solute carrier family 6, member 4) was cloned and localized to chromosome 17q11.1-q12 [98]. The gene contains a 630 amino acid open reading frame and consists of 15 exons [99, 100], including non-coding exons 1A and 1B [101]. The protein is one of several structurally similar sodium-dependent transporter proteins transferring specific substrates from the extracellular space to the cell cytoplasm. Two widespread polymorphisms of the 5-HTT gene have been described, a variable number of tandem repeats (VNTR) in intron 2 [99] and an insertion/deletion in the promoter region of the gene, named 5-HT transporter-linked polymorphic region (5-HTTLPR) [102]. Both polymorphisms are suggested to be involved in the expression of the serotonin transporter at the transcriptional level. There are significant allele-frequency differences for this polymorphism between Asiatic, African, American and European populations [103].

The polymorphism in intron 2 consists of a VNTR of nearly identical 17-bp segments. Although there are alleles with 9, 10, 11 and 12 repeats, the most common in all the populations studied are those with 10 and 12. For instance, in samples from northern Europeans the frequencies are 33,8 % and 66,2% respectively. The promoter variant in the 5-HT transporter gene, the 5-HTTLPR, consists of a 44 bp deletion/insertion in a repeat region of the promoter. This region consists of a variable number of repeated units of about 22 bp. This unit is repeated 14 or 16 times. The short (*s*) allele occurs with a frequency of approximately 50%; the long (*l*) form also has a frequency of 50% in samples of northern European ancestry. However, these allele frequencies differ widely in other populations [103]. In a population consisting of 31 French individuals with autism, (11 *ll*, 16 *sl* and 4 *ss*) 1,41 and 1,98 fold increase rate (Vmax) of platelet 5-HT uptake was observed in individuals with the *ll* genotype compared to the *ls* and *ss* genotypes, respectively [104]. However, levels of 5-HT in platelets and whole blood were not statistically significant across genotypes.

Several studies using the transmission/disequilibrium test to determine whether *SLC6A4* contributes to autism failed to reveal any reliable proof

of transmission of a specific 5-HTTLPR or VNTR allele [11, 105-110]. Evidence of preferential transmission of the short variant of 5-HTTLPR was found by Cook et al [110] and of the long allele by others [11, 105, 111]. In some studies, no evidence of association was reported [106, 107, 109, 112]. Kim et al [101], established a denser map of polymorphism after screening about 38 kb within or flanking the *SLC6A4* gene by direct sequencing of polymerase chain reaction (PCR) products from 10 unrelated autistic individuals. Twenty single nucleotide polymorphisms (SNPs) and seven simple sequence repeats (SSR) were typed in 115 autism trios. Although this study is not conclusive, some markers showed evidence of transmission disequilibrium to the level of $p = 0.005$.

## Degradation

Monoamine oxidase A (MAOA) seems to be the principal serotonin degrading enzyme. The gene is located on chromosome Xp11 [77]. Recent reports have shown that a low activity genetic variant of monoamine oxidase A (MAOA) is likely to be related to aggressive behavior, but only when paired with abusive experience in childhood. It also has been found that the low activity form of MAOA was associated with more adult symptoms of antisocial alcoholism than the high activity variant [113]. No studies related to autism have been carried out with this polymorphism (for more details see section 5 in Serotonergic innervations).

## Receptors

The 5-HT receptors families are summarized in Table 3. All the genes encoding the 5-HT receptors have been cloned. Some of them are intronless while other genes present several long introns. They are widely spread over the human genome and are present on chromosomes 1, 2, 3, 5, 6, 7, 10, 11, 13 and X. Interestingly, many of the 5-HT receptor genes appear to lie in or near autism linkage regions reported by various genome screens (Table 3).

5-HT receptors are grouped into seven classes ($5\text{-HT}_1$ to $5\text{-HT}_7$) based on their amino acid identity and functional characteristics. The members of each class have from 40% to 70% amino acid identity between them. This array of receptors offers an enormous signalling potential since alternative splicing and RNA editing increases further the variability of

proteins [5]. In addition, heterodimerization (e.g. 5-H$_{1B/1D}$) of the single receptors and the existence of multiple G proteins coupled to different receptors enlarge the capabilities of signal detection and phenotypic switching. The study of all these possible mechanisms and their role in vivo during physiological and pathological conditions is an enormous task. The distribution of 5-HT receptors in the brain has been mapped extensively by different techniques. The density of binding sites is especially high in limbic brain regions (see this excellent and detailed recent review [7]). The expression of 5-HT5A mRNA and other receptors in limbic structures and Purkinje cells, areas related to cognitive and emotional processes [86], is also suggestive of a link between serotonin and autism [87].

**Table 3. 5-Hidroxytryptamine receptors in humans.**

| RECEPTOR NAME | SUPERFAMILY | FAMILY | G PROTEIN EFFECTOR | GENE | CHROMOSOMAL* LOCALIZATION |
|---|---|---|---|---|---|
| 5-HT$_{1A}$ | GPCR[a] | 5-HT1 | $G_{i/o}$[b] | *HTR1A* | **5q**11.2-q13 |
| 5-HT$_{1B}$ | GPCR | 5-HT1 | $G_{i/o}$ | *HTR1B* | **6q**13 |
| 5-HT$_{1D}$ | GPCR | 5-HT1 | $G_{i/o}$ | *HTR1D* | **1p**34.3-36.3 |
| 5-ht$_{1E}$[c] | GPCR | 5-HT1 | $G_{i/o}$ | *HTR1E* | **6q**14-15 |
| 5-ht$_{1F}$ | GPCR | 5-HT1 | $G_{i/o}$ | *HTR1F* | **3p**11-p14.1 |
| 5HT$_{2A}$ | GPCR | 5-HT2 | $G_{q/11}$[d] | *HTR2A* | **13q**14-q21 |
| 5-HT$_{2B}$ | GPCR | 5-HT2 | $G_{q/11}$ | *HTR2B* | **2q**36.3-q37.1 |
| 5-HT$_{2C}$ | GPCR | 5-HT2 | $G_{q/11}$ | *HTR2C* | **Xq**24 |
| 5-HT$_3$ | Ligand gate ion channel | 5-HT3 | None | *HTR3* | 11q23.1-q23.2 |
| 5-HT$_4$ | GPCR | 5-HT4 | $G_s$[e] | *HTR4* | **5q**31-33 |
| 5-ht$_{5A}$ | GPCR | 5-HT5 | $G_{i/o}$ | *HTR5A* | **7q**36.1 |
| 5-ht$_{5B}$ | GPCR | 5-HT5 | No identified | *HTR5B* | **2q**11-q13 |
| 5-ht$_6$ | GPCR | 5-HT6 | $G_s$ | *HTR6* | **1p**35-36 |
| 5-HT$_7$ | GPCR | 5-HT7 | $G_s$ | *HTR7* | 10q23.3-24.3 |

[a] G-protein-couple receptor
[b] G-protein that inhibits cAMP formation
[c] Receptors with a lower case designate those that have not been demonstrated yet to function in native systems
[d] G-protein that increases the hydrolysis of inositol phosphates and elevates cytosolic $Ca^{++}$
[e] G-protein that promotes cAMP formation
* Chromosomal localization of 5-HT receptor genes. Bold and underline are chromosome regions linked to autism with highest score in published genome screen studies [33]

A distal region on chromosome 13q generated an overall lod score of 3.0 in the first four published genome screens of autistic sib pairs [114]. This region contains many genes, including the *HTR2A* that codes the serotonin receptor 5-$HT_{2A}$ (Table 3). Recently, Veenstra-VanderWeele et al [115] have carried out transmission disequilibrium studies with autism at three single polymorphisms (SNP) within *HTR2A*: the promoter –1438 G/A, intron 1 G/A and the 452 His/Tyr variant in exon 3. Although no evidence of positive association between autism and these particular SNPs was found, other polymorphisms within the 67.3 kb of the gene may yet be associated with the disorder.

Finally, post-transcriptional modifications of RNA also may contribute significantly to the complexity of the serotonin network. For instance, alternative splicing of mRNA precursors is an important factor generating protein diversity [116]. Furthermore, RNA editing also occurs at the 5-$HT_{2C}$ receptor to alter its activity [117]. This RNA editing refers to the conversion of adenosine to inosine in pre-mRNA to change triplet codons. The 5-$HT_{2C}$ receptor is edited at 5 different sites (named A, B, C, C' and D). Edited isoforms of these sites exhibit a significant reduction in the ability to activate the G-protein-coupled-receptor and have been associated with behavioral phenotypes. For instance, altered editing of the 5-$HT_{2C}$ receptor has been found in the prefrontal cortex of depressed suicide victims [118].

## FUTURE PERSPECTIVES

We conclude that the number of genes and their active isoforms involved in serotonin synthesis, release, transport, degradation and signal reception is extensive. This number might be increased, taking into account the genes interacting with them or playing a role in their regulation. As to their contribution to the autistic phenotype, we currently have no way of knowing whether these genes and their products exert primary effects or are part of a downstream pathway. Furthermore, estimates of the number of genes contributing to autism range up to 100, and each gene may have a small effect, making it difficult to detect an association with linkage studies or population genetic studies with current sample sizes (less than 1000 patients, for instance).

There are several ways in which investigators may be able to dissect this complexity from a genetic perspective, however. For instance, persons with hyperserotonemia might be screened separately from other autistic subjects to try to make a more homogeneous subgroup of patients. On the other hand, once sufficient numbers of patients have been evaluated in neuroimaging studies, it will be possible to create other phenotypically consistent subgroups of autistic children with brain structural characteristics that are strongly associated with autism (as the dorsolateral prefrontal cortex is with schizophrenia). In addition, all these subgroups could be assayed on microarrays to determine differential gene expression between patients and controls. These genes could then be explored further for polymorphisms. Finally, if we are lucky enough to discover even one autism susceptibility gene, we may be able to reconstruct the neurobiology of the disorder through exploration of related genes or neurochemical networks. It is possible that autism might arise by one or a few sets of signals affecting brain development in the prenatal and/or just postnatal period. Given the evidence we have provided, it seems likely that early disturbances of serotonin signaling will be involved in some fashion.

## ABBREVIATIONS

| | | |
|---|---|---|
| AADC | = | Amino acid decarboxylase |
| AD | = | Classic autism |
| AS | = | Asperger's syndrome |
| ATP | = | Adenosine-triphosphate |
| CDD | = | Childhood disintegrative disorder |
| CNS | = | Central nervous system |
| -COOH | = | Carboxyl group |
| DSM-IV | = | Diagnostic and Statistical Manual of Mental Disorder ($4^{th}$ edition) of the American Psychiatric Association (1994). |
| DZ | = | Dizygotic twins |
| 5-HT | = | 5-Hydroxytrypatmine |
| 5-HTP | = | 5-Hydroxytryptophan |
| 5-HTR | = | 5-Hydroytryptamine receptor |
| 5-HTT | = | 5-Hydroxytryptamine transporter |

| | | |
|---|---|---|
| 5-HTTLPR | = | 5-HT transporter-linked polymorphic region |
| MAO | = | Monoamine oxidase |
| MRI | = | Magnetic resonance imaging |
| MZ | = | Monozygotic twins |
| NADH | = | Reduced nicotinamide adenine dinucleotide |
| -OH | = | Hydroxyl group |
| PCR | = | Polymerase chain reaction |
| PDD | = | Pervasive development disorder |
| PDD-NOS | = | Pervasive development disorder–not otherwise specified |
| PET | = | Positron emission tomography |
| RTT | = | Rett's disorder |
| SNP | = | Single nucleotide polymorphisms |
| TH | = | Tryptophan hydroxylase |
| VNTR | = | Variable number tandem repeat |

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## REFERENCES

[1] Azmitia, E.C.; *Brain Res Bull,* **2001**, *56*, 413-424.
[2] Lauder, J.M.; Krebs, H.; *Dev Neurosci,* **1978**, *1*, 15-30.
[3] Veenstra-Vanderweele, J.; Anderson, G.M.; Cook, E.H., Jr.; *Eur J Pharmacol,* **2000**, *410*, 165-181.
[4] Young, S.N.; Leyton, M.; *Pharmacol Biochem Behav,* **2002**, *71*, 857-865.
[5] Pauwels, P.J.; *Biochem Pharmacol,* **2000**, *60*, 1743-1750.
[6] Hoyer, D.; Hannon, J.P.; Martin, G.R.; *Pharmacol Biochem Behav,* **2002**, *71*, 533-554.
[7] Barnes, N.M.; Sharp, T.; *Neuropharmacology,* **1999**, *38*, 1083-1152.
[8] Gillberg, C.; Wing, L.; *Acta Psychiatr Scand,* **1999**, *99*, 399-406.
[9] Kanner, L.; *Psychiatry Dig,* **1968**, *29*, 17-28.
[10] Anderson, G.M.; Freedman, D.X.; Cohen, D.J.; Volkmar, F.R.; Hoder, E.L.; Mcphedran, P.; Minderaa, R.B.; Hansen, C.R.; Young, J.G.; *J Child Psychol Psychiatry,* **1987**, *28*, 885-900.
[11] Yirmiya, N.; Pilowsky, T.; Nemanov, L.; Arbelle, S.; Feinsilver, T.; Fried, I.; Ebstein, R.P.; *Am J Med Genet,* **2001**, *105*, 381-386.

[12] Leboyer, M.; Philippe, A.; Bouvard, M.; Guilloud-Bataille, M.; Bondoux, D.; Tabuteau, F.; Feingold, J.; Mouren-Simeoni, M.C.; Launay, J.M.; *Biol Psychiatry,* **1999**, *45*, 158-163.
[13] Abramson, R.K.; Wright, H.H.; Carpenter, R.; Brennan, W.; Lumpuy, O.; Cole, E.; Young, S.R.; *J Autism Dev Disord,* **1989**, *19*, 397-407.
[14] Cook, E.H., Jr.; Arora, R.C.; Anderson, G.M.; Berry-Kravis, E.M.; Yan, S.Y.; Yeoh, H.C.; Sklena, P.J.; Charak, D.A.; Leventhal, B.L.; *Life Sci,* **1993**, *52*, 2005-2015.
[15] Mcdougle, C.J.; Naylor, S.T.; Cohen, D.J.; Aghajanian, G.K.; Heninger, G.R.; Price, L.H.; *Arch Gen Psychiatry,* **1996**, *53*, 993-1000.
[16] Gordon, C.T.; State, R.C.; Nelson, J.E.; Hamburger, S.D.; Rapoport, J.L.; *Arch Gen Psychiatry,* **1993**, *50*, 441-447.
[17] Mcdougle, C.J.; Naylor, S.T.; Cohen, D.J.; Volkmar, F.R.; Heninger, G.R.; Price, L.H.; *Arch Gen Psychiatry,* **1996**, *53*, 1001-1008.
[18] Whitaker-Azmitia, P.M.; *Brain Res Bull,* **2001**, *56*, 479-485.
[19] Moiseiwitsch, J.R.; *Crit Rev Oral Biol Med,* **2000**, *11*, 230-239.
[20] Medanic, M.; Gillette, M.U.; *J Physiol,* **1992**, *450*, 629-642.
[21] Ganguly, S.; Coon, S.L.; Klein, D.C.; *Cell Tissue Res,* **2002**, *309*, 127-137.
[22] Pacchierotti, C.; Iapichino, S.; Bossini, L.; Pieraccini, F.; Castrogiovanni, P.; *Front Neuroendocrinol,* **2001**, *22*, 18-32.
[23] Rind, H.B.; Russo, A.F.; Whittemore, S.R.; *Neuroscience,* **2000**, *101*, 665-677.
[24] Christenson, J.G.; Dairman, W.; Udenfriend, S.; *Proc Natl Acad Sci U S A,* **1972**. *69*, 343-347.
[25] Smalley, S.L.; Asarnow, R.F.; Spence, M.A.; *Arch Gen Psychiatry,* **1988**, *45*, 953-961.
[26] Ritvo, E.R.; Jorde, L.B.; Mason-Brothers, A.; Freeman, B.J.; Pingree, C.; Jones, M.B.; Mcmahon, W.M.; Petersen, P.B.; Jenson, W.R.; Mo, A.; *Am J Psychiatry,* **1989**, *146*, 1032-1036.
[27] Bolton, P.; Macdonald, H.; Pickles, A.; Rios, P.; Goode, S.; Crowson, M.; Bailey, A.; Rutter, M.; *J Child Psychol Psychiatry,* **1994**, *35*, 877-900.
[28] Szatmari, P.; Jones, M.B.; Zwaigenbaum, L.; Maclean, J.E.; *J Autism Dev Disord,* **1998**, *28*, 351-368.
[29] Folstein, S.; Rutter, M.; *J Child Psychol Psychiatry,* **1977**, *18*, 297-321.
[30] Ritvo, E.R.; Freeman, B.J.; Mason-Brothers, A.; Mo, A.; Ritvo, A.M.; *Am J Psychiatry,* **1985**, *142*, 74-77.
[31] Steffenburg, S.; Gillberg, C.; Hellgren, L.; Andersson, L.; Gillberg, I.C.; Jakobsson, G.; Bohman, M.; *J Child Psychol Psychiatry,* **1989**, *30*, 405-416.
[32] Bailey, A.; Le Couteur, A.; Gottesman, I.; Bolton, P.; Simonoff, E.; Yuzda, E.; Rutter, M.; *Psychol Med,* **1995**, *25*, 63-77.
[33] Folstein, S.E.; Rosen-Sheidley, B.; *Nat Rev Genet,* **2001**, *2*, 943-955.
[34] Filipek, P.A.; Accardo, P.J.; Baranek, G.T.; Cook, E.H., Jr.; Dawson, G.; Gordon, B.; Gravel, J.S.; Johnson, C.P.; Kallen, R.J.; Levy, S.E.; Minshew, N.J.; Ozonoff, S.; Prizant, B.M.; Rapin, I.; Rogers, S.J.; Stone, W.L.; Teplin, S.; Tuchman, R.F.; Volkmar, F.R.; *J Autism Dev Disord,* **1999**, *29*, 439-484.
[35] American Psychiatric Association DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed.: American Psychiatric Association, **1994**.

[36] Bailey, A.; Palferman, S.; Heavey, L.; Le Couteur, A.; *J Autism Dev Disord,* **1998**, *28*, 369-392.
[37] Piven, J.; *Curr Opin Neurobiol,* **1997**, *7*, 708-712.
[38] Amir, R.E.; Van Den Veyver, I.B.; Wan, M.; Tran, C.Q.; Francke, U.; Zoghbi, H.Y.; *Nat Genet,* **1999**, *23*, 185-188.
[39] Fombonne, E.; *Psychol Med,* **1999**, *29*, 769-786.
[40] Croen, L.A.; Grether, J.K.; Selvin, S.; *J Autism Dev Disord,* **2002**, *32*, 217-224.
[41] Chakrabarti, S.; Fombonne, E.; *Jama,* **2001**, *285*, 3093-3099.
[42] Jones, M.B.; Szatmari, P.; *Am J Med Genet,* **2002**, *114*, 558-565.
[43] Kemper, T.L.; Bauman, M.L.; *Mol Psychiatry,* **2002**, *7 Suppl 2*, S12-13.
[44] Kemper, T.L.; Bauman, M.; *J Neuropathol Exp Neurol,* **1998**, *57*, 645-652.
[45] Courchesne, E.; *Mol Psychiatry,* **2002**, *7 Suppl 2*, S21-23.
[46] Courchesne, E.; Karns, C.M.; Davis, H.R.; Ziccardi, R.; Carper, R.A.; Tigue, Z.D.; Chisum, H.J.; Moses, P.; Pierce, K.; Lord, C.; Lincoln, A.J.; Pizzo, S.; Schreibman, L.; Haas, R.H.; Akshoomoff, N.A.; Courchesne, R.Y.; *Neurology,* **2001**, *57*, 245-254.
[47] Sparks, B.F.; Friedman, S.D.; Shaw, D.W.; Aylward, E.H.; Echelard, D.; Artru, A.A.; Maravilla, K.R.; Giedd, J.N.; Munson, J.; Dawson, G.; Dager, S.R.; *Neurology,* **2002**, *59*, 184-192.
[48] Abell, F.; Krams, M.; Ashburner, J.; Passingham, R.; Friston, K.; Frackowiak, R.; Happe, F.; Frith, C.; Frith, U.; *Neuroreport,* **1999**, *10*, 1647-1651.
[49] Bailey, A.; Luthert, P.; Dean, A.; Harding, B.; Janota, I.; Montgomery, M.; Rutter, M.; Lantos, P.; *Brain,* **1998**, *121 (Pt 5)*, 889-905.
[50] Courchesne, E.; *Curr Opin Neurobiol,* **1997**, *7*, 269-278.
[51] Courchesne, E.; Yeung-Courchesne, R.; Press, G.A.; Hesselink, J.R.; Jernigan, T.L.; *N Engl J Med,* **1988**, *318*, 1349-1354.
[52] Pasqualetti, M.; Ori, M.; Castagna, M.; Marazziti, D.; Cassano, G.B.; Nardi, I.; *Neuroscience,* **1999**, *92*, 601-611.
[53] Carson, M.J.; Thomas, E.A.; Danielson, P.E.; Sutcliffe, J.G.; *Glia,* **1996**, *17*, 317-326.
[54] Grailhe, R.; Waeber, C.; Dulawa, S.C.; Hornung, J.P.; Zhuang, X.; Brunner, D.; Geyer, M.A.; Hen, R.; *Neuron,* **1999**, *22*, 581-591.
[55] Pierce, K.; Courchesne, E.; *Biol Psychiatry,* **2001**, *49*, 655-664.
[56] Baron-Cohen, S.; Ring, H.A.; Bullmore, E.T.; Wheelwright, S.; Ashwin, C.; Williams, S.C.; *Neurosci Biobehav Rev,* **2000**, *24*, 355-364.
[57] Kordower, J.H.; Piecinski, P.; Rakic, P.; *Brain Res Dev Brain Res,* **1992**, *68*, 9-15.
[58] Hariri, A.R.; Mattay, V.S.; Tessitore, A.; Kolachana, B.; Fera, F.; Goldman, D.; Egan, M.F.; Weinberger, D.R.; *Science,* **2002**, *297*, 400-403.
[59] Weidenfeld, J.; Newman, M.E.; Itzik, A.; Gur, E.; Feldman, S.; *Neuroendocrinology,* **2002**, *76*, 63-69.
[60] Hashimoto, S.; Inoue, T.; Koyama, T.; *Psychopharmacology (Berl),* **1996**, *123*, 182-186.
[61] Sibille, E.; Pavlides, C.; Benke, D.; Toth, M.; *J Neurosci,* **2000**, *20*, 2758-2765.
[62] Davis, M.; Shi, C.; *Ann N Y Acad Sci,* **1999**, *877*, 281-291.

[63] Raymond, G.V.; Bauman, M.L.; Kemper, T.L.; *Acta Neuropathol (Berl),* **1996**, *91*, 117-119.
[64] Saitoh, O.; Karns, C.M.; Courchesne, E.; *Brain,* **2001**, *124*, 1317-1324.
[65] Blatt, G.J.; Fitzgerald, C.M.; Guptill, J.T.; Booker, A.B.; Kemper, T.L.; Bauman, M.L.; *J Autism Dev Disord,* **2001**, *31*, 537-543.
[66] Sundstrom, E.; Kolare, S.; Souverbie, F.; Samuelsson, E.B.; Pschera, H.; Lunell, N.O.; Seiger, A.; *Brain Res Dev Brain Res,* **1993**, *75*, 1-12.
[67] Kontur, P.J.; Leranth, C.; Redmond, D.E., Jr.; Roth, R.H.; Robbins, R.J.; *Exp Neurol,* **1993**, *121*, 172-180.
[68] Levallois, C.; Valence, C.; Baldet, P.; Privat, A.; *Brain Res Dev Brain Res,* **1997**, *99*, 243-252.
[69] Shen, W.Z.; Luo, Z.B.; Zheng, D.R.; Yew, D.T.; *Pediatr Neurosci,* **1989**, *15*, 291-295.
[70] Takahashi, H.; Nakashima, S.; Ohama, E.; Takeda, S.; Ikuta, F.; *Brain Dev,* **1986**, *8*, 355-365.
[71] Toth, G.; Fekete, M.; *Acta Paediatr Hung,* **1986**, *27*, 221-226.
[72] Hedner, J.; Lundell, K.H.; Breese, G.R.; Mueller, R.A.; Hedner, T.; *Biol Neonate,* **1986**, *49*, 190-197.
[73] Chugani, D.C.; Muzik, O.; Behen, M.; Rothermel, R.; Janisse, J.J.; Lee, J.; Chugani, H.T.; *Ann Neurol,* **1999**, *45*, 287-295.
[74] Chugani, D.C.; Muzik, O.; Rothermel, R.; Behen, M.; Chakraborty, P.; Mangner, T.; Da Silva, E.A.; Chugani, H.T.; *Ann Neurol,* **1997**, *42*, 666-669.
[75] Chugani, D.C.; *Mol Psychiatry,* 2002, *7 Suppl 2*, S16-17.
[76] D'amato, R.J.; Blue, M.E.; Largent, B.L.; Lynch, D.R.; Ledbetter, D.J.; Molliver, M.E.; Snyder, S.H.; *Proc Natl Acad Sci U S A,* **1987**, *84*, 4322-4326.
[77] Grimsby, J.; Chen, K.; Wang, L.J.; Lan, N.C.; Shih, J.C.; *Proc Natl Acad Sci U S A,* **1991**, *88*, 3637-3641.
[78] Cases, O.; Vitalis, T.; Seif, I.; De Maeyer, E.; Sotelo, C.; Gaspar, P.; *Neuron,* **1996**, *16*, 297-307.
[79] Bennett-Clarke, C.A.; Leslie, M.J.; Lane, R.D.; Rhoades, R.W.; *J Neurosci,* **1994**, *14*, 7594-7607.
[80] Yan, W.; Wilson, C.C.; Haring, J.H.; *Brain Res Dev Brain Res,* **1997**, *98*, 177-184.
[81] Lauder, J.M.; *Ann N Y Acad Sci,* **1990**, *600*, 297-313; discussion 314.
[82] Lidov, H.G.; Molliver, M.E.; *Brain Res Bull,* **1982**, *8*, 389-430.
[83] Mitchell, J.B.; Iny, L.J.; Meaney, M.J.; *Brain Res Dev Brain Res,* **1990**, *55*, 231-235.
[84] Yan, W.; Wilson, C.C.; Haring, J.H.; *Brain Res Dev Brain Res,* **1997**, *98*, 185-190.
[85] Gross, C.; Zhuang, X.; Stark, K.; Ramboz, S.; Oosting, R.; Kirby, L.; Santarelli, L.; Beck, S.; Hen, R.; *Nature,* **2002**, *416*, 396-400.
[86] Pasqualetti, M.; Ori, M.; Nardi, I.; Castagna, M.; Cassano, G.B.; Marazziti, D.; *Brain Res Mol Brain Res,* 1998, *56*, 1-8.
[87] Marazziti, D.; *Biol Psychiatry,* **2002**, *52*, 143.
[88] Kowlessur, D.; Kaufman, S.; *Biochim Biophys Acta,* **1999**, *1434*, 317-330.

[89] Nielsen, D.A.; Goldman, D.; Virkkunen, M.; Tokola, R.; Rawlings, R.; Linnoila, M.; *Arch Gen Psychiatry,* **1994**, *51*, 34-38.
[90] Hong, C.J.; Tsai, S.J.; Wang, Y.C.; *Schizophr Res,* **2001**, *49*, 59-63.
[91] Sumi-Ichinose, C.; Ichinose, H.; Takahashi, E.; Hori, T.; Nagatsu, T.; *Biochemistry,* **1992**, *31*, 2229-2238.
[92] Chatelin, S.; Wehrle, R.; Mercier, P.; Morello, D.; Sotelo, C.; Weber, M.J.; *Brain Res Mol Brain Res,* **2001**, *97*, 149-160.
[93] Swoboda, K.J.; Hyland, K.; Goldstein, D.S.; Kuban, K.C.; Arnold, L.A.; Holmes, C.S.; Levy, H.L.; *Neurology,* **1999**, *53*, 1205-1211.
[94] Peter, D.; Finn, J.P.; Klisak, I.; Liu, Y.; Kojis, T.; Heinzmann, C.; Roghani, A.; Sparkes, R.S.; Edwards, R.H.; *Genomics,* **1993**, *18*, 720-723.
[95] Shih, J.C.; Ridd, M.J.; Chen, K.; Meehan, W.P.; Kung, M.P.; Seif, I.; De Maeyer, E.; *Brain Res,* **1999**, *835*, 104-112.
[96] Cook, E.H., Jr.; Leventhal, B.L.; Freedman, D.X.; *Biol Psychiatry,* **1988**, *24*, 488-491.
[97] Katsui, T.; Okuda, M.; Usuda, S.; Koizumi, T.; *J Autism Dev Disord,* **1986**, *16*, 69-76.
[98] Ramamoorthy, S.; Bauman, A.L.; Moore, K.R.; Han, H.; Yang-Feng, T.; Chang, A.S.; Ganapathy, V.; Blakely, R.D.; *Proc Natl Acad Sci U S A,* **1993**, *90*, 2542-2546.
[99] Lesch, K.P.; Balling, U.; Gross, J.; Strauss, K.; Wolozin, B.L.; Murphy, D.L.; Riederer, P.; *J Neural Transm Gen Sect,* **1994**, *95*, 157-162.
[100] Bradley, C.C.; Blakely, R.D.; *J Neurochem,* **1997**, *69*, 1356-1367.
[101] Kim, S.J.; Cox, N.; Courchesne, R.; Lord, C.; Corsello, C.; Akshoomoff, N.; Guter, S.; Leventhal, B.L.; Courchesne, E.; Cook Jr, E.H.; *Mol Psychiatry,* **2002**, *7*, 278-288.
[102] Heinrich, R.; Cromarty, S.I.; Horner, M.; Edwards, D.H.; Kravitz, E.A.; *Proc Natl Acad Sci U S A,* **1999**, *96*, 2473-2478.
[103] Gelernter, J.; Cubells, J.F.; Kidd, J.R.; Pakstis, A.J.; Kidd, K.K.; *Am J Med Genet,* **1999**, *88*, 61-66.
[104] Anderson, G.M.; Gutknecht, L.; Cohen, D.J.; Brailly-Tabard, S.; Cohen, J.H.; Ferrari, P.; Roubertoux, P.L.; Tordjman, S.; *Mol Psychiatry,* **2002**, *7*, 831-836.
[105] Klauck, S.M.; Poustka, F.; Benner, A.; Lesch, K.P.; Poustka, A.; *Hum Mol Genet,* **1997**, *6*, 2233-2238.
[106] Maestrini, E.; Lai, C.; Marlow, A.; Matthews, N.; Wallace, S.; Bailey, A.; Cook, E.H.; Weeks, D.E.; Monaco, A.P.; *Am J Med Genet,* **1999**, *88*, 492-496.
[107] Persico, A.M.; Militerni, R.; Bravaccio, C.; Schneider, C.; Melmed, R.; Conciatori, M.; Damiani, V.; Baldi, A.; Keller, F.; *Am J Med Genet,* **2000**, *96*, 123-127.
[108] Tordjman, S.; Gutknecht, L.; Carlier, M.; Spitz, E.; Antoine, C.; Slama, F.; Carsalade, V.; Cohen, D.J.; Ferrari, P.; Roubertoux Et, A.; *Molecular Psychiatry,* **2001**, *6*, 434-439.
[109] Betancur, C.; Corbex, M.; Spielewoy, C.; Philippe, A.; Laplanche, J.L.; Launay, J.M.; Gillberg, C.; Mouren-Simeoni, M.C.; Hamon, M.; Giros, B.; Nosten-Bertrand, M.; Leboyer, M.; *Mol Psychiatry,* **2002**, *7*, 67-71.

[110] Cook, E.H., Jr.; Courchesne, R.; Lord, C.; Cox, N.J.; Yan, S.; Lincoln, A.; Haas, R.; Courchesne, E.; Leventhal, B.L.; *Mol Psychiatry,* **1997**, *2*, 247-250.

[111] Tordjman, S.; Gutknecht, L.; Carlier, M.; Spitz, E.; Antoine, C.; Slama, F.; Carsalade, V.; Cohen, D.J.; Ferrari, P.; Roubertoux, P.L.; Anderson, G.M.; *Mol Psychiatry,* **2001**, *6*, 434-439.

[112] Zhong, N.; Ye, L.; Ju, W.; Brown, W.T.; Tsiouris, J.; Cohen, I.; *Neurogenetics,* **1999**, *2*, 129-131.

[113] Hill, E.M.; Stoltenberg, S.F.; Bullard, K.H.; Li, S.; Zucker, R.A.; Burmeister, M.; *Psychiatr Genet,* **2002**, *12*, 143-153.

[114] Barrett, S.; Beck, J.C.; Bernier, R.; Bisson, E.; Braun, T.A.; Casavant, T.L.; Childress, D.; Folstein, S.E.; Garcia, M.; Gardiner, M.B.; Gilman, S.; Haines, J.L.; Hopkins, K.; Landa, R.; Meyer, N.H.; Mullane, J.A.; Nishimura, D.Y.; Palmer, P.; Piven, J.; Purdy, J.; Santangelo, S.L.; Searby, C.; Sheffield, V.; Singleton, J.; Slager, S.; Et Al.; *Am J Med Genet,* **1999**, *88*, 609-615.

[115] Veenstra-Vanderweele, J.; Kim, S.J.; Lord, C.; Courchesne, R.; Akshoomoff, N.; Leventhal, B.L.; Courchesne, E.; Cook, E.H., Jr.; *Am J Med Genet,* **2002**, *114*, 277-283.

[116] Caceres, J.F.; Kornblihtt, A.R.; *Trends Genet,* **2002**, *18*, 186-193.

[117] Burns, C.M.; Chu, H.; Rueter, S.M.; Hutchinson, L.K.; Canton, H.; Sanders-Bush, E.; Emeson, R.B.; *Nature,* **1997**, *387*, 303-308.

[118] Gurevich, I.; Tamir, H.; Arango, V.; Dwork, A.J.; Mann, J.J.; Schmauss, C.; *Neuron,* **2002**, *34*, 349-356.

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# ANTIVIRAL ACTIVITIES OF POLYSACCHARIDES FROM NATURAL SOURCES

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**ABSTRACT**: The ever increasing resistance of human pathogens to current anti-infective agents is a serious medical problem, leading to the need to develop novel antibiotic prototype molecules. In the case of viruses, the search for antiviral agents involves additional difficulties, particularly due to the nature of the infectious viral agents. Thus, many compounds that may cause the death of viruses are also very likely to injure the host cell that harbours them. Natural products are increasingly appreciated as leads for drug discovery and development. Screening studies have been carried out in order to find antiviral agents from natural sources, and the occurrence of antiviral activity in extracts of plants, marine organisms and fungi is frequent. The evidence indicates that there may be numerous potentially useful antiviral phytochemicals in nature, waiting to be evaluated and exploited. In addition, other plants, not previously utilized medicinally, may also reveal antivirals. Among natural antiviral agents, recent investigations have reconsidered the interest of phyto-polysaccharides, which act as potent inhibitors of different viruses. This chapter will illustrate a variety of antiviral polysaccharides from natural sources since 1990, with the aim of making this matter more accessible to drug development

## INTRODUCTION

Infectious diseases account for one-third of all deaths worldwide. Although the last decade has yielded significant advances in the treatment of infectious diseases, new therapies for viral, fungal, bacterial and parasitic infections are needed [1].

Viruses are responsible for a large proportion of the morbidity and mortality experienced worldwide. These infectious agents consist of a core genome of nucleic acid, DNA or RNA (nucleoid) contained in a protein shell (capsid) and sometimes surrounded by a lipoprotein membrane (envelope). Some genera of viruses are known to cause human infections and include the following: adeno, pox and herpesviruses:

herpes simplex virus type 1 and 2, varicella-zoster, cytomegalovirus, Epstein-Barr virus; papillomaviruses (papillomas, cancer); parvoviruses (erythema infectiosum); DNA-viruses (e.g., hepatitis B); (+/-) RNA viruses: reo- and rotavirus; (-) RNA viruses: influenza, parainfluenza, measles, respiratory syncytial, vesicular stomatitis, rabies virus and togaviruses (rubella, yellow fever); retroviruses (e.g., human immunodeficiency virus, the causative agent of acquired immunodeficiency syndrome); and other several RNA viruses such as arenaviruses (lymphocytic choriomeningitis), bunyaviruses (encephalitis), coronaviruses (upper respiratory infections) and picornaviruses (poliomyelitis, diarrhoea). Most successful anti-infective agents inhibit essential steps in metabolic processes required by the pathogen but absent in the host. However, viruses cannot replicate independently. They must enter cells and use the cellular energy-generating, DNA- or RNA-replicating and protein-synthesizing pathways of the host to achieve viral replication. For this reason, in the case of viruses the search for antiviral agents is very hard since metabolic differences are not available: viruses utilize the host's protein synthesis leaving only some aspects of nucleic acid synthesis and macromolecule processing distinct from the host's metabolism.

The identification of a retrovirus, human immunodeficiency virus, as the causative agent of acquired immunodeficiency syndrome, the steadily increasing incidence of various viral infections caused by viruses in immunodeficient patients, e,g., herpes simplex virus, varicella-zoster virus, cytomegalovirus and Epstein-Barr virus, the increasing evidence for the pathogenic role of a number of viruses, e.g., human papilloma virus in the pathogenesis of primary hepatocellular carcinoma, and the socioeconomic impact of viral infections of the respiratory (influenza, adeno-, corona- and rhinoviruses) and gastrointestinal tract (rotaviruses) have all been important factors in boosting the search for new antiviral agents and new modalities of antiviral chemotherapy. Although many compounds with potent antiviral activity in cell cultures and in experimental animals have been detected, at present only several molecules and α-interferon have been approved by the health authorities for therapy of viral infections in humans. Among these antiviral substances are several natural compounds isolated from plants used in traditional medicine, including polysaccharides [2-4].

Polysaccharides (PS) constitute a structurally diverse class of biological macromolecules with a wide range of physicochemical properties, which are the basis for different applications in the broad field of pharmacy and medicine [5-8]. Several hundred natural PS are currently known and provide one of the richest and oldest reservoirs of structurally and functionally diverse biopolymers. PS constitute important members of the family of industrial water-soluble polymers. Besides the classical applications of these biopolymers in industry and pharmaceutical practice, the relatively new field of pharmacologically active polymers will be discussed. PS from plants and other natural sources have long been known to exert antitumour, immunomodulatory, anticoagulant and other types of biological activity, including antiviral activity. The importance of the plant kingdom as a source of new antiviral substances will be illustrated by presenting a survey on natural-derived antiviral PS.

In recent years, natural and synthetic polymers of a carbohydrate nature were proved to be selective inhibitors of several enveloped viruses, including such human pathogens as human immunodeficiency virus, herpes simplex virus, human cytomegalovirus, influenza virus and respiratory syncytial virus. Recent efforts have been directed at the development of polysaccharidic molecules as selective inhibitors of different classes of viruses [9,10]. In this search, the following molecular targets were envisaged: for DNA viruses in general, the viral DNA polymerase; for herpes simplex virus and varicella-zoster virus, the viral DNA polymerase via a specific phosphorylation by the viral 2'-deoxythymidine kinase; for (+/-) RNA and (-) RNA viruses, S-adenosylhomocysteine hydrolase, a key enzyme in transmethylation reactions required for the maturation of viral mRNA; for retroviruses, reverse transcriptase as an initiator of virus replication and/or cell transformation; and for several enveloped viruses (e.g., retro-, herpes- and rhabdoviruses), virus adsorption to the outer cell membrane. This review deals with antiviral PS compiled from several sources during the past decade. Under the respective titles, we offer some brief descriptions of the most important global viral infections that are caused either by individual viruses or by groups of related viruses. They are restricted to those generally considered important in human and veterinary medicine and about which reports on active antiviral PS are recorded in the literature.

## HUMAN IMMUNODEFICIENCY VIRUS

Acquired immunodeficiency syndrome (AIDS) is a pandemic immunosuppressive disease which results in life-threatening opportunistic infections and malignancies. In 1996, the number of AIDS infections was 23 million and 1.5 million people (half of them in Africa) were killed by this disease worldwide. In order to combat human immunodeficiency virus (HIV), the causative agent of AIDS, enormous amounts of time and money have been dedicated to research into compounds which can be developed as therapeutic agents. HIV is a lentivirus which replicates within critical cells of the immune system, particularly CD4 T-cells and monocyte/macrophages, leading to a progressive loss of helper T-cells and profound immunosuppression. This condition is known as AIDS. Without treatment, the CD4 T-lymphocytes of an infected patient are reduced markedly so that the patient is susceptible to a wide range of opportunistic infections, and ultimately dies. Since this retrovirus, designated HIV, has been clearly identified as the primary cause of this disease, numerous compounds, also including plant-derived substances, have been evaluated for their inhibitory effects on HIV replication *in vitro*. Furthermore, many plant products are being used by patients with AIDS in some countries without any scientific proof that they possess anti-HIV activity.

Several reviews on natural products for the chemotherapy of HIV infections have been recorded in the literature [11-19]. Carbohydrates were among the natural molecules capable of treating HIV infection. Various PS, including sulphated PS, have been found to be anti-HIV active substances of many antivirally active plant extracts, all of which are medicinal plants used in traditional medicine as anti-infectives. For example, a PS (MAR-10) was isolated from the aqueous extract of the plant *Hyssop officinalis* L., which was found to inhibit HIV-1 replication as demonstrated by the inhibition of HIV-1 p24 antigen and syncytia formation [20]. From *Aspalathos linearis* Burm., a popular medicinal plant in South Africa, Europe and Japan, a PS with strong anti-HIV activity was isolated [21,22]. This compound almost completely inhibited the binding of HIV-1 to MT-4 cells. The major sugar components of this purified PS were found to be glucose, galactose, mannose and xylose. Premanathan *et al.* [23,24] investigated the PS extracted from *Rizophora apiculata* Blume and *Rizophora mucronata* Poir. in cell culture systems

for their activity against human and simian immunodeficiency viruses (SIV). Both compounds inhibited HIV-1, HIV-2 or SIV strains in various cell cultures and assay systems. It was found that these PS consisted mainly of neutral sugars and uronic acids.

In a screening programe of Thai plants for anti-HIV activity, it was found that 114 extracts of 81 species belonging to 32 families showed activity [25]. Of the effective samples, the extracts of *Merremia peltata* (L.) Merrill were the most active. Most of the anti-HIV agents were found to be anionic PS. Furthermore, sulphated derivatives of paramylon, a (1-3)-β-D-glucan from *Euglena gracilis* Klebs. significantly inhibited the cytopathic effect of HIV-1 and HIV-2 [26], while two naturally occurring PS isolated from the tropical climbing shrub *Ipomoea cairica* (L.) Sweet were found to strongly inhibit replication of HIV-1 *in vitro* [27].

According to De Clercq [28], the replicative cycle of HIV comprises several steps that could be considered adequate targets for chemotherapeutical intervention: virus entry, viral adsorption, virus-cell fusion, reverse (RNA → DNA) transcription, proviral DNA integration, viral (DNA → RNA) transcription (transactivation), viral (mRNA → protein) translation, virus release, viral assembly, budding and maturation. The most notable difference between HIV replication and other retroviruses is the intricate control of HIV gene expression by viral and cellular factors [29]. Possible mechanisms by which HIV kills infected cells include the formulation of multinucleate syncytia, cytopathic components within the virions themselves, and interaction between viral envelope proteins and the CD4 molecule on the cell surface. Three HIV enzymes are essential to the life cycle of the virus: HIV reverse transcriptase is crucial for viral replication; HIV protease processes viral polyproteins into functional enzymes and structural proteins, thereby facilitating maturation and infectivity of the virion particles; HIV integrase mediates HIV integration into the host chromosome. The chemotherapeutic strategies have therefore been focused on the development of inhibitors of these retroviral enzymes. Recently, it has been reported that biological membranes arising from HIV-induced cell fusion, as well as syncytium formation between infected and non-infected cells are interesting approaches in research for a new HIV therapy [30].

Natural sulphated PS such as dextran sulphate, pentosan sulphate, chondroitin sulphate and heparin have long been recognized as effective anti-HIV agents [31-35]. These compounds target the interaction between

the viral envelope glycoprotein gp120 and the CD4 receptor and, as a consequence, they inhibit not only virus adsorption to the cells but also virus-induced syncytium (giant cells) formation [36-42]. The inhibitory effect of dextran sulphate and its congeners on viral binding, viral replication and syncytium formation appears to be mediated by a specific interaction with the V3 region of gp120. In addition, sulphated PS may also directly interfere with the binding of HIV particles to the heparin sulphate proteoglycan of the cell surface, whether or not this process occurs independently of, or cooperatively with, the viral envelope CD4 receptor interaction.

This class of antiviral agents exhibits several unique properties that are not shared by other currently known antiviral agents: remarkable broad-spectrum antiviral activity against HIV-1, HIV-2 and a series of other enveloped viruses; the ability to inhibit syncytium formation between HIV-infected and normal CD4 T-lymphocytes, a mechanism that drastically enhances HIV infectivity and a low induction of viral drug-resistance [43]. It should be noted that sulphated PS owe their anti-HIV activity to the presence of the sulphate groups, which in turn are responsible for the inhibition of virus-cell binding. Dextran is a high-molecular-weight PS consisting predominantly of $\alpha$ $(1 \rightarrow 6)$ linked D-glucose units. Dextrans can differ in chain length and in degree of branching. The branching occurs via $\alpha$ $(1 \rightarrow 3)$ and $\alpha$ $(1 \rightarrow 4)$ branch points. Dextran fractions of different average molecular weight were obtained by partial hydrolysis. These different fractions were sulphated to obtain dextran sulphates of different molecular weight.

Busso and Resnick [44] investigated the effects of three molecular weight ranges of dextran sulphate on five different HIV isolates. The higher molecular weight range of dextran sulphate showed the most potent activity as assessed by a quantitative syncytium formation assay. However, dextran sulphate is poorly adsorbed after oral administration and upon intravenous administration produces thrombocytopenia, so that there is little, if any, evidence for the *in vivo* efficacy of this compound and more generally of sulphated PS against HIV infection [45]. However, double-blind placebo-controlled clinical pilot trials in advanced HIV disease are being conducted with these compounds [46].

In summary, these compounds offer great promise as candidate drugs for the chemotherapy of HIV infections, and novel sulphated PS which

have proved to be potent and selective inhibitors of HIV *in vitro* are being chemically synthesized [47-52].

On the other hand, although several groups have reported that sulphated PS are potent and selective inhibitors of HIV, their therapeutic application is however limited by their anticoagulant activity. In view of possible improvements in therapeutic potential, a number of heparin derivatives with reduced anticoagulant activity were studied for their inhibitory activity in an HIV-dependent syncytium formation assay [53]. The most pronounced anti-HIV activity was observed with 30% succinylated standard heparin and 100% succinylated low molecular weight heparin. Similarly, and in order to increase the ratio of anti-HIV activity to anticoagulant activity, selectively substituted sulphated PS with OH and/or COOH groups were chemically prepared [54,55]. This group comprises a family of heterogeneous carbohydrates composed of long, unbranched PS modified, for example, by sulphations and acetylations. The acetylated derivatives are more active as inhibitors of HIV-induced giant-cell formation. Furthermore, several soluble derivatized dextrans with different percentages of carbomethyl, benzylamide and sulphonate/sulphate groups were also evaluated for possible inhibitory effects on HIV-1 infection, and from the results obtained, their use as anti-HIV therapeutic agents can be proposed [56].

Other natural sources of anti-HIV sulphated PS include algae and other marine organisms. Sulphated PS have been found in a variety of marine animals, plants and microorganisms [57,58]. Aqueous extracts of many marine invertebrates have exhibited some activity in the National Cancer Institute's primary screen for anti-HIV cytopathicity [59]. Carrageenans and fucoidan are sulphated PS extracted from red seaweed and brown algae, which have shown potent inhibitory activity against different viruses including HIV. For example, fucoidan, a complex sulphated PS from the alga *Fucus vesiculosus* L. was found to inhibit HIV *in vitro*, presumably through a direct interaction of the PS with the HIV binding site of the target cells [60]. The aqueous-soluble extracts from this alga, a common littoral alga of the coast of the Northern Atlantic, the Pacific Ocean and the Baltic Sea, inhibited the activity of HIV reverse transcriptase enzyme as well as HIV-induced syncytium formation. Béress *et al.* [61] reported a new procedure for the isolation of the anti-HIV PS from this marine alga.

A novel sulphated PS named calcium spirulan has been isolated as an antiviral principle from *Spirulina platensis* (Nordst.) Geitl., a blue-green

alga growing in some African and Central and South American lakes rich in salts [62-65]. Chemical analyses of calcium spirulan suggest that it contains rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid and galacturonic acid. Other sulphated PS from marine sources have demonstrated antiviral activities against HIV, blocking viral replication in cell culture without any toxicity to the host cells. Examples include sulphated cell-wall PS from the Mediterranean red alga *Asparagopsis armata* Harvey [66], from the marine microalga *Cochlodinium polykrikoides* Blooms [67], fucose, the main component sugar of the PS isolated from the brown alga *Sargasum horneri* (Turner) C. Agardh [68] and *Sargasum muticum* (Yendo) Fensholt. [69], sulphated PS from Brazilian marine algae such as *Laminaria abyssalis* AB Joly & EC Oliveira [70], and from tropical marine algae of the Codiaceae family [71], and new sulphated β-galactans from the marine clam species *Meretrix petechialis* Lamarck [72]. Other natural sources of anti-HIV sulphated PS include fungi such as *Ganoderma lucidum* (Fr.) Karst. [73] and *Lentinus edodes* (Lem.) mycelia [74].

Inhibition of HIV-reverse transcriptases (HIV-RT) is currently considered a useful approach in the prophylaxis and intervention of AIDS and natural products have not as yet been extensively explored as inhibitors of this enzyme [75,76]. Furthermore, the mechanisms of action of sulphated PS may not only be due to the inhibition of virus cell adhesion, but also to the inhibition of HIV-RT [13,15].

## HERPES SIMPLEX VIRUS

Herpes infections are ubiquitous. Approximately, 16-35%, 40-80% and >90% of the population are sero-positive for, or infected by herpes simplex virus type I (HSV-1), herpes simplex virus type II (HSV-2) and varicella-zoster virus, respectively. More alarmingly, over the past decade, the incidence and severity of infections caused by HSV have increased due to the growth in number of immunocompromised patients produced by aggressive chemotherapy regimens, expanded organ transplantation and a greater occurrence of HIV infections. Unfortunately, prolonged therapies with acyclovir, the most successful antiherpetic drug, have resulted in some undesirable complications and also induced the emergence of drug-resistant viruses. With this change in disease pattern,

and the increase in drug use frequency, acyclovir-resistant HSV infections have emerged.

Infections with HSV range from simple cold sores and fever blisters to severe central nervous system disorders. Development of effective antiviral medications has made prompt recognition important in primary care practice. Appropiate therapy can significantly reduce both the medical and psychosocial ramifications of herpes infections and can greatly improve the quality of life of many patients [77]. Thus, there is an urgent need for novel anti-HSV agents, especially those with a different mode of action from acyclovir. For the past decades, in addition to a variety of synthetic antiviral drugs with different molecular targets, a large number of phytochemicals have been recognized to control infections caused by viruses belonging to the Herpesviridae family. Ethnopharmacological screenings of medicinal plants from all over the world have led to the selection of several extracts which are active against herpes viruses.

Among natural antiviral agents, recent investigations have revived the interest in phyto-PS, which act as potent inhibitors of different enveloped viruses, including members of Herpesviridae. For example, a neutral PS extracted from *Sclerotium glucanicum* Halleck., namely scleroglucan, revealed promising antiviral activity [78]. These PS are composed of a β-1,3-linked glucopyranose backbone with single β-1,6-linked glucopyranosyl branches every third subunit. The blockage on HSV infection takes place during the very early phases of the viral multiplication cycle, since the greatest inhibitory effect occurred when it was added during the attachment step.

As a result of the intensive search for antiviral substances from medicinal plants, antiviral activity against HSV was found in extracts from *Cedrela tubiflora* Bertoni. leaves [79], from *Prunella vulgaris* L., a perennial plant commonly found in China, the British Isles and Europe [80] and from *Trichilia glabra* L. leaves [81]. Phytochemical studies indicate that these plants contain anionic PS as active constituents which may inhibit HSV by competing for cell receptors as well as by some unknown mechanisms after the virus has penetrated the cells. Furthermore, the *in vitro* antiviral activity demonstrated by extracts of the medicinal plant *Achyrocline flaccida* (Weinm.) D.C. on HSV-1 is exerted early during viral replication, essentially during viral adsorption to host

cells [82]. A bioguided purification process indicated that negatively charged PS were responsible for this antiviral activity.

Among natural antiviral agents of a carbohydrate nature, recent investigations have also revived interest in sulphated phyto-PS such as carrageenans and fucoidan from seaweed. Sea algae represent a very interesting source of potential antiviral compounds, particularly the water-soluble sulphated PS which are abundant constituents of the cell wall. These compounds act as potent inhibitors of different enveloped viruses, including members of Herpesviridae, and their activity is linked to the anionic features of the molecule which hinder the attachment of viral particles to host cells. For example, carrageenans are sulphated galactans that can be extracted from certain red seaweeds. They comprise a broad range of structures and can be divided into two families: the κ-family, defined by the presence of a C4-sulphate group on the β-D-unit, and the λ-family, characterized by the sulphate on C2 and constituted by all the variations of the λ-structures [83].

Investigations have detected antiviral activity in a significant number of algae from various marine environments such as California, United Kingdom, the Mediterranean, India and Japan [84]. In the course of a screening study on the biological properties of natural marine products, diverse carrageenans isolated from the red seaweed *Gigartina skottsbergii* Setch. et Gard. have proved to be potent inhibitors of HSV-1 and –2 *in vitro* [85]. Mode of action studies are consistent with the carrageenans having a major effect on the attachment of virus to the cells. Adsorption of HSV-1 is primarily mediated by the envelope glycoprotein gC, which binds to heparan sulphate residues present on the proteoglycans on the surface of target cells. Antiherpetic activity was directly correlated to the amount of α-D-galactose 2,6-disulphate residues in the natural carrageenans. Carrageenans were also isolated from Chilean samples of *Stenogramme interrupta* (C. Ag.) Mont., a red seaweed from California [86].

In the course of screening for antiviral properties in sulphated PS isolated from marine algae of the South American coast, Carlucci *et al.* [87] reported the antiviral activity of mannans and xylogalactans isolated from the red seaweed *Nothogenia fastigiata* Turner (Lam.), and a sulphated galactan from *Pterocladia capillacea* (Gmelim) Barnet & Thuset. HSV-1 and –2 were the most sensitive viruses of these compounds due to an inhibition of virus binding. Structural analysis of

two xylomannans extracted from *Nothogenia fastigiata* was carried out [88,89]. The results are consistent with the general pattern previously reported for other xylomannans of the same system, α(1 → 3)-linked D-mannans 2- and 6-sulphated and with single stubs of β(1 → 2)-linked D-xylose, but one of the new samples contains a significant amount of 2,6-disulphated units. The presence of sulphate groups in the molecule was essential to the antiviral properties of these PS [90].

The antiviral activity of sulphated PS is known to increase with molecular weight or the degree of sulphation. However, sulphated PS are generally endowed with anticoagulant properties that may hamper their usefulness as antiviral drugs. Duarte *et al.* [91] reported the inhibitory effect of sulphated galactans from the marine alga *Bostrychia montagnei* Harvey. The results of the activated partial thromboplastin time assay and the thrombine time assay indicated that three natural sulphated PS have very low anticoagulant activity, confirming that there is no relation between the antiviral and anticoagulant properties.

Anti-HSV sulphated PS have also been isolated from other seaweeds such as rhamnan sulphate from *Monostroma latissimum* Wittrock [92], and calcium spirulan from the blue-green alga *Spirulina platensis* [62]. Lee *et al.* [93] initiated studies into the structure-activity relationships of calcium spirulan. Calcium ion binding with the anionic part of the molecule was replaced with various metal cations. Replacement of the calcium ion with sodium and potassium ions maintained anti-HSV-1 activity while divalent and trivalent cations reduced the activity. The cell wall sulphated PS of the red microalga *Porphyridium* spp. Honey exhibited impressive antiviral activity against HSV-1 and –2 both *in vitro* (cell culture) and *in vivo* (rats and rabbits) [94]. It seems that this PS is able to inhibit viral infection by preventing adsorption of the virus into the host cells and/or by inhibiting the production of new viral particles inside the host cells [95]. Novel fucan sulphates with anti-HSV activity were also isolated from the hot water extract of an edible brown alga *Sargassum horneri* [96] and from the brown seaweed *Leathesia difformis* (Linnaeus) Areschong [97].

Other natural sources of anti-HSV PS include fungi, e.g., various protein bound PS isolated from *Ganoderma lucidum*, a basidiomycetous fungus used to treat human diseases in oriental folk medicine [98]. The possible mode of antiviral activity of these PS seems to be related to their binding with HSV-specific glycoproteins responsible for attachment and

penetration, impeding the complex interactions of viruses with cell plasma membranes [99-101]. Furthermore, different semisynthetic PS were evaluated for their inhibitory effect on *in vitro* replication of HSV-1 and –2 [102-104]. Some neutral and negatively charged carbohydrates were able to inhibit viral infection by interfering mainly with the adsorption process.

The *in vitro* and *in vivo* effects of sulphated PS were also investigated against the pseudorabies virus (Suid HSV-1), the most notable of which is porcine HSV which has a well-documented history of epizootic infections, especially in Europe [105]. *In vitro* experiments revealed that sulphated PS significantly reduced the number of lytic plaques, but *in vivo* only heparin protected mice against the pseudorabies virus.

## CYTOMEGALOVIRUS

In terms of their biological and pathogenetic properties, the herpes viruses fall naturally into several subfamily groupings, including cytomegalovirus (CMV), although detailed classification is at present premature. Nevertheless the CMV clearly constitute a group of their own with internal consistency. Human CMV is, together with HSV-1, one of the agents responsible for opportunistic infections in HIV-infected people.

As can be seen in the present review, sulphated PS show antiviral activitiy against enveloped viruses *in vitro*. It has been suggested that these antiviral mechanisms depend on their polyanionic properties, which interact with the positively charged amino acid sequence of viral envelope glycoproteins. Natural sulphated PS can act as potent inhibitors of CMV. The cell wall mucilages of seaweeds are known to be rich in sulphated PS with potential anti-CMV activity. Recently, Blinkova *et al.*[106] reviewed information on *Spirulina platensis*, a blue-green alga with diverse biological activity. Preparations obtained from this alga have been found to be active against several enveloped viruses, including CMV. It was revealed that calcium spirulan, a sulphated PS isolated from *Spirulina platensis*, selectively inhibited the penetration of the virus into host cells [62]. Retention of molecular conformation by chelation of calcium ion with sulphate groups may be indispensable to its antiviral effect. The antiviral activity of a polysaccharidic fraction obtained from another red seaweed, *Nothogenia fastigiata*, was also reported [107]. Its mode of

action against CMV could be ascribed to an inhibitory effect on virus adsorption.

Other natural sources of sulphated PS include brown algae. A sulphated polysaccharide with potent anti-CMV activity was isolated from the brown alga *Sargassum horneri* [68]. Time-of-addition experiments suggested that it inhibited not only the initial stages of viral infection, such as attachment to and penetration into host cells, but also later replication stages after virus penetration. Fractions of fucoidan, another polysaccharide isolated from the brown seaweed *Leathesia difformis* were also found to be selective antiviral agents against human CMV [97]. These compounds offer great promise as candidate drugs for the chemotherapy of CMV infections, and novel sulphated PS are being chemically prepared [108].

PS from terrestrial plants have also been reported as anti-CMV agents. For example, PS from three plant species, *Astragalus brachycentrus* D. C., *Astragalus echidnaeformis* Sirjaev and *Sterculia urens* Roxb., which are devoid of *in vitro* antiviral activity, were evaluated in mouse models of murine CMV infections [109]. Treatment with the compounds needed to be started one day prior to virus inoculation for maximum protective benefit. Treatments starting after virus inoculation were ineffective. The mannose-specific plant PS from the orchid species *Cymbidium hybrid* Cym., *Epipactis helleborine* (L.) Crantz. and *Listera ovata* (L.) R.Br. Svenska are potent and selective inhibitors of human CMV *in vitro* [110]. They presumably interact at the level of virion fusion with the target cell.

## PAPILLOMAVIRUSES

Papillomaviruses are members of the Papopavirus family and are distinguished from other members by virtue of their association with a variety of warts in different parts of the body. Some types have been implicated in genital warts and cervical carcinomas, while others seem to be associated with other distinctive warts elsewhere. No generally effective control is available, although potentially dangerous lesions can be removed by cryosurgery or laser treatment. However, some medicinal plant preparations have been reported to be beneficial; conceivably these may work by promoting healing or stimulating immune responses, rather than directly inhibiting the virus.

Natural high-molecular weight sulphated or sulphonated PS, such as cellulose sulphate and dextran sulphate, may be useful non-toxic microbicidal compounds that are active against a variety of sexually-transmitted disease agents, including bovine papillomavirus type 1 and human papilloma virus type 11 and type 40 [111].

## HEPATITIS VIRUSES

Hepatitis B virus (HBV) is widespread throughout human populations, specially in Asia and Africa, and it has been estimated that over 200 million carriers exist, some of whom are eventually expected to develop liver carcinoma or cirrhosis. HBV shows a strict tropism for liver hepatocytes in which it displays a protected replication with resultant foci of liver necrosis. The virus is a member of the Hepadnaviridae, along with several other species, and it replicates by a mechanism which appears to be unique to this family. In contrast, hepatitis A virus is a picornavirus and the hepatitis D agent appears to be a viroid-like RNA enclosed within a hepatitis B capsid, and consequently depends upon its association with the HBV for its spread and survival. Control may be effected by passive immunization (with hyperimmune globulin) or by various types of vaccines which are currently being developed and improved. Specific chemotherapy has not been consistently successful, but in some countries (e.g., India and China), plant extracts have provided some success.

Among natural antiviral agents of carbohydrate nature, sulphated PS such as λ and κ carrageenans showed a potent inhibitory effect on the replication of HBV in the human hepatoma cell line PLC/PRF/5 [112]. The two types of carrageenans resulted in concentration-dependent reduction of HBV-antigen expression and HBV infectivity, emerging as promising candidates for chemotherapy of acute hepatitis.

## ROTAVIRUSES

Human rotavirus (HRV), a member of the Reoviridae, is a non-enveloped virus which is the major etiologic agent of severe dehydrating gastroenteritis in children worldwide. For the treatment of rotavirus gastroenteritis, intravenous fluid administration has been used successfully for dehydration from diarrhoea. However, in the case of

severe inpatients and immunocompromised hosts who are suffering from prolonged diarrhoea and fever, virus-specific treatment will be necessary if possible. Several compounds, biomaterials and plant extracts have been found to be inhibitory for HRV of some species *in vitro*. For example, extracts of *Stevia rebaudiana* Bertoni, a family member of Chrysanthemum originating from Paraguay in South America, inhibited the replication of all four serotypes of HRV *in vitro* [113]. Binding assays with radiolabeled purified viruses indicated that the inhibitory mechanism of this plant extracts is the blockage of virus binding. The inhibitory components of *Stevia rebaudiana* were found to be heterogeneous anionic PS with different ion charges.

## INFLUENZA VIRUSES

Influenza continues to have a significant impact on public health. Annually, 20,000 deaths and 100,000 hospitalizations are attributed to "flu" in the United States alone. Airborne transmission, facile viral mutation, vaccine shortages, and actual and perceived side effects and limitations of both vaccines and prophylactic drugs contribute to the drive for new therapies and preventive medicines for influenza. Research during the last fifteen years has elucidated many of the mechanisms by which the influenza virus invades, captures and mobilizes the replication capabilities of a host cell, providing new targets in the search for antiviral treatments. For the past decades, besides a variety of synthetic antiviral drugs with different molecular targets, a large number of phytochemicals have been recognized to control infections caused by the influenza virus. Serkedjieva *et al.* [114] reported the antiviral activity from a lyophilized infusion from flowers of *Sambucus nigra* L., aerial parts of *Hypericum perforatum* L. and roots of *Saponaria officinalis* L. The preparations contain PS which could be responsible for their antiviral properties.

Reports on the anti-influenza virus activity of natural PS are recorded in the literature. From a pine cone extract of *Pinus parviflora* Sieb. et Zucc., an acidic polysaccharide was isolated [115,116]. This compound showed significant inhibition of both the viral protein synthesis in infected cells and virion-associated RNA-dependent RNA polymerase activity. In animal models *in vivo*, most mice inoculated intranasally or intracerebrally with lethal doses of the influenza virus A/WSN/33 died within 12 days. However, the infectivity of the virus that had been

preincubated with a polysaccharide prepared from cones of *Pinus parviflora* was significantly reduced [117]. In 1991, Sakagami *et al.*[118] reviewed the potential antiviral efficacy of natural polysaccharidic-related materials isolated from the pine cone extracts.

Sulphated PS, potent antiviral agents, were also evaluated *in vitro* as inhibitors of influenza virus replication [119]. The fact that the sulphated PS are inhibitory to some myxoviruses and retroviruses but not to others seems to depend on the composition of the amino acid sequences of the viral envelope glycoproteins that are involved in virus-cell binding and fusion [120].

As can be seen in the present review, other natural sources of antiviral sulphated PS include the marine environment. Sulphated and non-sulphated PS with anti-influenza activity have been isolated from marine sources such as the green marine alga *Ulva lactuca* L. [121], the Japanese sea alga *Crenomytilus grayanus* Dunkei. [122,123], the blue-green alga *Spirulina platensis* [106], the red seaweed *Nothogenia fastigiata* [107] and the marine microalga *Cochlodinium polykrikoides* [67]. Novel marine PS which have proved to be potent and selective inhibitors of the influenza virus *in vitro* are being chemically synthesized [124,125].

## MEASLES VIRUS

The measles virus (MV) is a paramyxovirus which has been associated with several chronic diseases. Since the advent of mass immunization campaigns, the incidence of measles has decreased dramatically in many developed countries. This is not the case elsewhere, however, where the virus still takes its toll in large numbers, especially in malnourished or immunocompromised individuals. Secondary bacterial infections are prevalent in these populations. Although most infections are relatively short-lived and innocuous in healthy individuals, encephalitis can develop in approximately 0.1% of cases; hence vaccination is desirable. Following respiratory infection, the virus readily invades local lymphoid tissues and gains access to the blood, from where it disseminates widely in the body. There has not been much demand for chemotherapy; vaccination seems to be the choice. No really effective antiviral has been evaluated, although some plant compounds have been shown to inhibit MV replication effectively, such as PS from the blue-green alga *Spirulina platensis* [62].

## RABIES VIRUS

Rabies has long been recognized as a scourge of livestock with occasional and often fatal intrusions into humans and their pets. Initially the virus gains access to muscle, sensory organs or skin, and there replicates in local unmyelinated (sensory) nerve fibers. Many layers of epithelial cells are susceptible to the virus but the principal target is the unprotected neuron. The brain is where most damage is done, resulting in the familiar psychomotor disturbances. In addition, the virus spreads into salivary glands (by axoplasmic flow again), from where it is secreted from the asinar mucous cells and transmitted to the victim. The objective of much research on the rabies virus has been the development of better vaccines and therapeutic measures, some of them from natural sources. For example, different natural polymeric carbohydrates inhibited rabies virus infection in chicken-embryo-related cells by interfering with the virus adsorption process [126]. The charge density and the polymeric backbone of the molecules seem to play a role in influencing the antiviral properties, whereas other features such as the sugar moieties do not appear to be relevant.

## TOGAVIRUS

Rubella is the sole member of the Rudivirus genus in the Togavirus family. The virus is transmitted by the respiratory route, and multiplies in upper respiratory tissues from where it gains access to the blood, accompanied sometimes by a characteristic rash and other symptoms. The infection may be more severe in pregnant women. Vaccination programmes are in place in many countries, and the belief is that the virus will eventually be brought under control. Although there seems to be no apparent role or need for specific antiviral therapy, reports on the anti-rubella activity of natural products and extracts have been reported [57]. For example, the natural carbohydrate scleroglucan, three sulphate derivatives and dextran sulphate were investigated for their inhibitory effect on rubella virus infection on Vero cells [127]. The results indicated that PS blocked a step in virus replication subsequent to virus attachment, such as internalisation and/or uncoating.

## BUNYAVIRUS

Sandfly fever Sicilian virus (SFSV) is a Phlebovirus, a member of the Bunyaviridae family, which cause acute, incapaciting but self-limiting diseases in man, and which have had a major impact in Europe, the Middle East and Africa. SFSV is a virus closely related to other viruses which are considerably more pathogenic both in humans and animals, such as the African Swine fever virus (ASFV) and the Crimean-Congo haemorrhagic fever virus (CCHFV).

The ASFV is a highly contagious bunyavirus infection of pigs which is clinically indistinguishable from the unrelated hog cholera virus. Since 1957, epidemics have been recorded intermittently in several European countries bordering the Mediterranean, in the Caribbean, and in Brazil, and more recently in other European countries and parts of Africa. Although pigs appear to be the only species infected naturally, the virus can be adapted to grow in a variety of other cell types and experimentally infects goats and rabbits. In view of this, it seems worthwhile to consider the possibility of its natural persistence in animals other than swine and man. Transmission of ASFV usually occurs through the respiratory route. Following inhalation, the virus invades and attacks local lymph nodes and the endothelium of blood vessels, with the result that high titers of virus circulate in the blood and lymph, and eventually in various secretory fluids. Attempts have been made to produce vaccines, but without much success. In any case, the virus mutates frequently in the wild and cross-protection of animals is not complete. Since the virion contains several enzyme activities, and the DNA can probably code for more in the infected cell, the possibility of specific chemotherapy, along the lines of antiherpes chemicals, should be profitable.

Several studies have been undertaken to develop drugs which could be used for treatment of fever infections caused by viruses belonging to the Bunyaviridae family, including natural PS [128,129]. Antiviral activity was estimated by the reduction of the cytophatic effect of SFSV on infected Vero cells. Several PS, such as carrageenans, fucoidan, dextran sulphate and pentosan polysulphate caused a concentration-dependent reduction in the virus yield. Fabregas *et al.* [130] screened several extracts from marine microalgae for *in vitro* inhibition of the replication of ASFV. That this inhibition could be due to sulphated PS was suggested because the same pattern of viral inhibition was obtained by using exocellular

extracts from microalgae enriched in these compounds and/or dextran sulphate of high molecular weight.

Other natural PS should be considered for the potential treatment of significant human infections caused by bunyaviruses. The mouse model of Punta Toro virus, a Phlebovirus member of the Bunyaviridae family, is a model for studying the treatment of Rift Valley fever infection. Tragacanthin PS from *Astragalus brachycentrus* and *Astragalus echidnaeformis* plants caused reduction in mortality, liver infection scores, liver and spleen virus titers, and serum transaminases in treated mice [131].

## POLIOVIRUSES

Poliomyelitis is not the scourge that it once was, thanks to improved hygienic practices which have effectively blocked the spread of wild-type polioviruses, especially in developed countries, and thanks to the widespread use of vaccines. The viruses, like other enteroviruses, are transmitted by the fecal-oral route; following ingestion, they replicate in various cell types in the pharynx and small intestine (they are quite stable to stomach acids) and gain access to the circulation. From there, they disseminate and occasionally gain access to the central nervous system, where they may produce the characteristic lesions leading to poliomyelitis.

Control of virus spread accompanied by vaccination continues to provide the best means of alleviating poliomyelitis. The use of antivirals seems to have less application than for many other viral infections, although the poliovirus continues to serve as a useful laboratory model for antiviral screening. The plant kingdom and seaweeds may be a source of potentially useful and interesting antiviral compounds against polioviruses. For example, anti-poliovirus activity was detected in a polysaccharidic-rich fraction from extracts of several Korean seaweeds [132], and from the leaves of the Meliaceae tree *Trichilia glabra* [81].

## OTHER VIRUSES

Other compounds of carbohydrate nature with antiviral activity against other viruses have been isolated from natural sources. Several compounds

belonging to different classes of sulphated PS were evaluated for their inhibitory effects on the replication of the arenavirus Junin and Tacaribe in Vero cells [133,107]. Very potent and selective inhibitors were the sulphated PS dextran sulphate, λ-carrageenan, fucoidan, heparin and pentosan polysulphate. Examples also include aloe polymannose, a high mannose purified from the *Aloe barbadensis* Miller plant, which showed activity in enhancing antibody titers against coxsackievirus B3-induced myocarditis in murine models of the disease [134], and fungal PS which are active *in vivo* in Sendai virus infection [135].

Although relatively little work has been done on natural antivirals against plant viruses, several reports concerning antiviral activity against plant virus infection have been recorded; for example, yeast mannans with antiviral activity against the tobacco mosaic virus infection in tobacco plants [136], and lichenan PS from Iceland moss which exhibited antiviral activity against the potato viruses [8].

## ABBREVIATIONS

PS = Polysaccharides
AIDS = Acquired Immunodeficiency syndrome
HIV = Human immunodeficiency virus
SIV = Simian immunodeficiency virus
HIV-RT = HIV-reverses transcriptases
HSV = Herpes Simplex Virus
CMV = Cytomegalovirus
HBV = Hepatitis B virus
HRV = Human rotavirus
MV = Measles virus
SFSV = Sandfly fever Sicilian virus
ASFV = African Swine fever virus
CCHFV = Crimean-Congo haemorrhagic fever virus

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## REFERENCES


[1] Harris, B. D.; Adal, K. A.; Tomecki, K. J. *Dermatol Clin* **2000**, *18*, 31-35.
[2] Vlietinck, A. J.; Vanden Berghe, D. A. *J Ethnopharmacol* **1991**, *32*, 141-153.
[3] Cragg, G. M.; Boyd, M. R.; Cardellina, J. H.; Newman, D. J.; Snader, K. M. *Ciba Found Symp* **1994**, *185*, 178-190.
[4] Vlietinck, A. J.; DeBruyne, T.; VandenBerghe, D. *Cur Org Chem* **1997**, *1*, 307-344.
[5] Franz, G. *Planta Med* **1989**, *55*, 493-497.
[6] Tian, G.; Feng, Y. *Zhongguo Zhong Yao Za Zhi* **1995**, *20*, 441-444.
[7] Ovodov, I. *Bioorg Khim* **1998**, *24*, 483-501.
[8] Olafsdottir, E. S.; Ingolfsdottir, K. *Planta Med* **2001**, *67*, 199-208.
[9] De Clercq, E. *Microbiologica* **1990**, *13*, 165-178.
[10] De Clercq, E. *Adv Virus Res* **1993**, *42*, 1-55.
[11] Pauwels, R.; Schols, D. *Verh K Acad Geneeskd Belg* **1991**, *53*, 629-655.
[12] Houghton, P. J. *Trans R Soc Trop Med Hyg* **1996**, *90*, 601-604.
[13] Ng, T. B.; Huang, B.; Fong, W. P.; Yeung, H. W. *Life Sci* **1997**, *61*, 933-949.
[14] Vlietinck, A. J.; De Bruyne, T.; Apers, S.; Pieters, L. A. *Planta Med* **1998**, *64*, 97-109.
[15] Matthee, G.; Wright, A. D.; Konig, G. M. *Planta Med* **1999**, *65*, 493-506.
[16] De Clercq, E. *Med Res Rev* **2000**, *20*, 323-349.
[17] Jung, M.; Lee, S.; Kim, H.; Kim, H. *Curr Medl Chem* **2000**, *7*, 649-661.
[18] Premanathan, M.; Rajendran, S.; Ramanathan, T.; Kathiresan, K.; Nakashima, H.; Yamamoto, N. *Indian J Med Res* **2000**, *112*, 73-77.
[19] Wu, J. A.; Attele, A. S.; Zhang, L.; Yuan, C. S. *Am J Chin Med* **2001**, *29*, 69-81.
[20] Gollapudi, S.; Sharma, H. A.; Aggarwal, S.; Byers, L. D.; Ensley, H. E.; Gupta, S. *Biochem Biophys Res Commun* **1995**, *210*, 145-151.
[21] Nakano, M.; Itoh, Y.; Mizuno, T.; Nakashima, H. *Biosci Biotechnol Biochem* **1997**, *61*, 267-271.
[22] Nakano, M.; Nakaswima, H.; Itoh, Y. *Leukemia* **1997**, *11*, 128-130.
[23] Premanathan, M.; Kathiresan, K.; Yamamoto, N.; Nakashima, H. *Biosci Biotechnol Biochem* **1999**, *63*, 1187-1191.
[24] Premanathan, M.; Arakaki, R.; Izumi, H.; Kathiresan, K.; Nakano, M.; Yamamoto, N.; Nakashima, H. *Antiviral Res* **1999**, *44*, 113-122.
[25] Yamamoto, T.; Takahashi, H.; Sakai, K.; Kowithayakorn, T.; Koyano, T. *Nat Med* **1997**, *51*, 541-546.
[26] Koizumi, N.; Sakagami, H.; Utsumi, A.; Fujinaga, S.; Takeda, M.; Asano, K.; Sugawara, I.; Ichikawa, S.; Kondo, H.; Mori, S. *Antiviral Res* **1993**, *21*, 1-14.
[27] Schroder, H. C.; Merz, H.; Steffen, R.; Muller, W. E.; Sarin, P. S.; Trumm, S.; Schulz, J.; Eich, E. *Z Naturforsch* **1990**, *45*, 1215-1221.
[28] De Clercq, E. *Clin Microbiol Rev* **1995**, *8*, 200-239.
[29] Wells, K. H.; Poiesz, B. J. *J Am Acad Dermatol* **1990**, *22*, 1175-1195.
[30] Raulin, J. *Prog Lipid Res* **2002**, *41*, 27-65.

[31] Weiler, B. E.; Schroder, H. C.; Stefanovich, V.; Stewart, D.; Forrest, J. M.; Allen, L. B.; Bowden, B. J.; Kreuter, M. H.; Voth, R.; Muller, W. E. *J Gen Virol* **1990**, *71*, 1957-1963.
[32] Bagasra, O.; Whittle, P.; Heins, B.; Pomerantz, R. J. *J Infect Dis* **1991**, *164*, 1082-1090.
[33] Jagodzinski, P. P.; Wiaderkiewicz, R.; Kurzawski, G.; Kloczewiak, M.; Nakashima *Virology* **1994**, *202*, 735-745.
[34] Thormar, H.; Balzarini, J.; Debyser, Z.; Witvrouw, M.; Desmyter, J.; De Clercq, E. *Antiviral Res* **1995**, *27*, 49-57.
[35] Sakagami, H.; Satoh, K.; Ida, Y.; Koyama, N.; Premanathan, M.; Arakaki, R.; Nakashima, H.; Hatano, T.; Okuda, T.; Yoshida, T. *Life Sci* **1999**, *66*, 595-611.
[36] Callahan, L. N.; Phelan, M.; Mallinson, M.; Norcross, M. A. *J Virol* **1991**, *65*, 1543-1550.
[37] Harrop, H. A.; Rider, C. C.; Coombe, D. R. *Biochem Soc Trans* **1992**, *20*, 163S.
[38] Gilbert, M.; Brigido, L.; Muller, W. E.; Hansen, J. E.; Ezekowitz, R. A.; Mills, J. *J Virol Methods* **1993**, *42*, 1-12.
[39] Meshcheryakova, D.; Andreev, S.; Tarasova, S.; Sidorova, M.; Vafina, M.; Kornilaeva, G.; Karamov, E.; Khaitov, R. *Mol Immunol* **1993**, *30*, 993-1001.
[40] Okada, T.; Patterson, B. K.; Gurney, M. E. *Biochem Biophys Res Commun* **1995**, *209*, 850-858.
[41] Jeon, K. J.; Katsuraya, K.; Kaneko, Y.; Mimura, T.; Uryu, T. *Macromolecules* **1997**, *30*, 1997-2001.
[42] Harrop, H. A.; Rider, C. C. *Glycobiology* **1998**, *8*, 131-137.
[43] Luscher, M. M. *Antiviral Chem Chemother* **2000**, *11*, 249-259.
[44] Busso, M. E.; Resnick, L. *Antimicrob Agents Chemother* **1990**, *34*, 1991-1995.
[45] Hiebert, L. M.; Wice, S. M.; Jaques, L. B.; Williams, K. E.; Conly, J. M. *J Lab Clin Med* **1999**, *133*, 161-170.
[46] Montaner, J. S.; Gill, J.; Singer, J.; Raboud, J.; Arseneau, R.; McLean, B. D.; Schechter, M. T.; Ruedy, J. *J Acquir Immune Defic Syndr Hum Retrovirol* **1996**, *12*, 153-157.
[47] Rider, C. C.; Coombe, D. R.; Harrop, H. A.; Hounsell, E. F.; Bauer, C.; Feeney, J.; Mulloy, B.; Mahmood, N.; Hay, A.; Parish, C. R. *Biochemistry* **1994**, *33*, 6974-6980.
[48] Clayette, P.; Moczar, E.; Mabondzo, A.; Martin, M.; Toutain, B.; Marce, D. *Aids Res Hum Retroviruses* **1996**, *12*, 63-69.
[49] Larack, S. A.; Melton, D. J.; Lewis, M. S. *Glycoconjugate J* **1998**, *15*, 697-712.
[50] Leydet, A.; Moullet, C.; Roque, J. P.; Witvrouw, M.; Pannecouque, C.; Andrei, G.; Snoeck, R.; Neyts, J.; Schols, D.; De Clercq, E. *J Med Chem* **1998**, *41*, 4927-4932.
[51] Chen, C.; Huang, X.; Zhou, J.; Xu, H.; Jiang, Y.; Zhu, G.; Sun, D. *Yao Xue Xue Bao* **1998**, *33*, 264-268.
[52] Nishimura, S. I.; Kai, H.; Shinada, K.; Yoshida, T.; Tokura, S.; Kurita, K. *Carbohydr Res* **1998**, *306*, 427-433.

[53] Lopalco, L.; Ciccomascolo, F.; Lanza, P.; Zoppetti, G.; Caramazza, I.; Leoni, F. *Aids Res Hum Retroviruses* **1994**, *10*, 787-793.
[54] Barzu, T.; Level, M.; Petitou, M.; Lormeau, J. C.; Choay, J.; Schols, D.; Baba, M. *J Med Chem* **1993**, *36*, 3546-3555.
[55] Herold, B. C.; Gerber, S. I.; Polonsky, T.; Belval, B. J.; Shaklee, P. N.; Holme, K. *Virology* **1995**, *206*, 1108-1116.
[56] Seddiki, N.; Mbemba, E.; Letourneur, D.; Ylisastigui, L.; Benjouad, A.; Saffar *Biochim Biophys Acta* **1997**, *1362*, 47-55.
[57] Witvrouw, M.; De Clercq, E. *Gen Pharmacol* **1997**, *29*, 497-511.
[58] Schaeffer, D. J.; Krylov, V. S. *Ecotoxicol Environ Saf* **2000**, *45*, 208-227.
[59] Beutler, J. A.; Mckee, T. C.; Fuller, R. W.; Tischler, M.; Cardellina, J. H.; Snader, K. M. *Antiviral Chem Chemother* **1993**, *4*, 167-172.
[60] McClure, M. O.; Whitby, D.; Patience, C.; Gooderham, N. J.; Bradshaw, A.; Cheingsong, T. *Antiviral Chem Chemother* **1991**, *2* , 149-156.
[61] Beress, A.; Wassermann, O.; Tahhan, S.; Bruhn, T.; Beress, L.; Kraiselburd, E. N.; Gonzalez, L. V.; de Motta, G. E.; Chavez, P. I. *J Nat Prod* **1993**, *56*, 478-488.
[62] Hayashi, T.; Hayashi, K.; Maeda, M.; Kojima, I. *J Nat Prod* **1996**, *59*, 83-87.
[63] Hayashi, K.; Hayashi, T.; Kojima, I. *Aids Res Hum Retroviruses* **1996**, *12*, 1463-1471.
[64] Ayehunie, S.; Belay, A.; Baba, T. W.; Ruprecht, R. M. *J Acquir Immune Defic Syndr Hum Retrovirol* **1998**, *18*, 7-12.
[65] Lee, J. B.; Hayashi, T.; Hayashi, K.; Sankawa, U.; Maeda, M.; Nemoto, T.; Nakanishi, H. *J Nat Prod* **1998**, *61*, 1101-1104.
[66] Haslin, C.; Lahaye, M.; Pellegrini, M.; Chermann, J. C. *Planta Med* **2001**, *67*, 301-305.
[67] Hasui, M.; Matsuda, M.; Okutani, K.; Shigeta, S. *Int J Biol Macromol* **1995**, *17*, 293-297.
[68] Hoshino, T.; Hayashi, T.; Hayashi, K.; Hamada, J.; Lee, J. B.; Sankawa, U. *Biol Pharm Bull* **1998**, *21*, 730-734.
[69] Nakamura, H.; Ohnuki, N.; Sadamasu, K.; Sekine, H.; Tanaka, J.; Okada, Y.; Okuyama, K. *Nat Med* **1994**, *48*, 173-179.
[70] Romanos, M.; Andrada-Serpa, M. J.; Dos, S.; Ribeiro, A.; Yoneshigue-Valentin, Y.; Costa, S. S.; Wigg, M. D. *Cancer Invest* **2002**, *20*, 46-54.
[71] Siddhanta, A. K.; Shanmugam, M. *J Indian Chem Soc* **1999**, *76*, 323-334.
[72] Amornrut, C.; Toida, T.; Imanari, T.; Woo, E. R.; Park, H.; Linhardt, R.; Wu, S. J.; Kim, J. *Carbohydr Res* **1999**, *321*, 121-127.
[73] Kim, H. W.; Shim, M. J.; Cho, E. C.; Kim, B. K. *Arch Pharm Res* **1997**, *20*, 425-431.
[74] Suzuki, H.; Iiyama, K.; Yoshida, O.; Yamazaki, S.; Yamamoto, N.; Toda, S. *Agric Biol Chem* **1990**, *54*, 479-487.
[75] Broder, S.; Mitsuya, H.; Yarchoan, R.; Pavlakis, G. N. *Ann Intern Med* **1990**, *113*, 604-618.

[76] Tan, G. T.; Pezzuto, J. M.; Kinghorn, A. D.; Hughes, S. H. *J Nat Prod* **1991**, *54*, 143-154.
[77] Nadelman, C. M.; Newcomer, V. D. *Postgrad Med* **2000**, *107*, 189-200.
[78] Marchetti, M.; Pisani, S.; Pietropaolo, V.; Seganti, L.; Nicoletti, R.; Degener, A.; Orsi, N. *Planta Med* **1996**, *62*, 303-307.
[79] Craig, M. I.; Benencia, F.; Coulombie, F. C. *Fitoterapia* **2001** , *72*, 113-119.
[80] Xu, H. X.; Lee, S. H.; Lee, S. F.; White, R. L.; Blay, J. *Antiviral Res* **1999**, *44*, 43-54.
[81] Benencia, F.; Courreges, M. C.; Coulombie, F. C. *Fitoterapia* **1997**, *68*, 173-176.
[82] Garcia, G.; Cavallaro, L.; Broussalis, A.; Ferraro, G.; Martino, V.; Campos, R. *Planta Med* **1999**, *65*, 343-346.
[83] Carlucci, M. J.; Ciancia, M.; Matulewicz, M. C.; Cerezo, A. S.; Damonte, E. B. *Antiviral Res* **1999**, *43*, 93-102.
[84] Hudson, J. B.; Kim, J. H.; Lee, M. K.; Hong, Y. K.; DeWreede, R. E. *Pharm Biol* **1999**, *37*, 300-306.
[85] Carlucci, M. J.; Pujol, C. A.; Ciancia, M.; Noseda, M. D.; Matulewicz, M. C.; Damonte, E. B.; Cerezo, A. S. *Int J Biol Macromol* **1997**, *20*, 97-105.
[86] Caceres, P. J.; Carlucci, M. J.; Damonte, E. B.; Matsuhiro, B.; Zuniga, E. A. *Phytochemistry* **2000**, *53*, 81-86.
[87] Carlucci, M. J.; Scolaro, L. A.; Errea, M. I.; Matulewicz, M. C.; Damonte, E. B. *Planta Med* **1997**, *63*, 429-432.
[88] Pujol, C. A.; Coto, C. E.; Damonte, E. B. *Rev Argent Microbiol* **1995**, *27*, 91-98.
[89] Kolender, A. A.; Pujol, C. A.; Damonte, E. B.; Matulewicz, M. C.; Cerezo, A. S. *Carbohydr Res* **1997**, *304*, 53-60.
[90] Damonte, E. B.; Matulewicz, M. C.; Cerezo, A. S.; Coto, C. E. *Chemotherapy* **1996**, *42*, 57-64.
[91] Duarte, M. E.; Noseda, D. G.; Noseda, M. D.; Tulio, S.; Pujol, C. A.; Damonte, E. B. *Phytomedicine* **2001**, *8*, 53-58.
[92] Lee, J. B.; Hayashi, K.; Hayashi, T.; Sankawa, U.; Maeda, M. *Planta Med* **1999**, *65*, 439-441.
[93] Lee, J. B.; Srisompom, P.; Hayashi, K.; Tanaka, T.; Sankawa, U.; Hayashi, T. *Chem Pharm Bull* **2001**, *49*, 108-110.
[94] Huheihel, M.; Ishanu, V.; Tal, J.; Arad, S. M. *J Biochem Biophys Methods* **2002**, *50*, 189-200.
[95] Huleihel, M.; Ishanu, V.; Tal, J.; Arad, S. *J Appl Phycol* **2001**, *13*, 127-134.
[96] Preeprame, S.; Hayashi, K.; Lee, J. B.; Sankawa, U.; Hayashi, T. *Chem Pharm Bull* **2001**, *49*, 484-485.
[97] Feldman, S. C.; Reynaldi, S.; Stortz, C. A.; Cerezo, A. S.; Damonte, E. B. *Phytomedicine* **1999**, *6*, 335-340.
[98] Eo, S. K.; Kim, Y. S.; Lee, C. K.; Han, S. S. *J Ethnopharmacol* **1999**, *68*, 175-181.

[99] Eo, S. K.; Kim, Y. S.; Lee, C. K.; Han, S. S. *J Ethnopharmacol* **2000**, *72*, 475-481.

[100] Oh, K. W.; Lee, C. K.; Kim, Y. S.; Eo, S. K.; Han, S. S. *J Ethnopharmacol* **2000**, *72*, 221-227.

[101] Kim, Y. S.; Eo, S. K.; Oh, K. W.; Lee, C.; Han, S. S. *J Ethnopharmacol* **2000**, *72*, 451-458.

[102] Marchetti, M.; Pisani, S.; Pietropaolo, N.; Seganti, L.; Nicoletti, R.; Orsi, N. *J Chemother* **1995**, *7*, 90-96.

[103] Tian, G. Y.; Li, S. T.; Song, M. L.; Zheng, M. S.; Li, W. *Yao Xue Xue Bao* **1995**, *30*, 107-111.

[104] Kolender, A. A.; Pujol, C. A.; Damonte, E. B.; Cerezo, A. S.; Matulewicz, M. C. *Anal Asoc Quim Arg* **1998**, *86*, 304-311.

[105] Ramos-Kuri, M.; Kretschmer, R. R.; Espinosa-Larios, E. L.; Aguilar-Setien, A. *Arch Invest Med* **1990**, *21*, 29-33.

[106] Blinkova, L. P.; Gorobets, O. B.; Baturo, A. P. *Zh Mikrobiol Epidemiol Immunobiol* **2001**, *5*, 114-118.

[107] Damonte, E.; Neyts, J.; Pujol, C. A.; Snoeck, R.; Andrei, G.; Ikeda, S.; Witvrouw, M.; Reymen, D.; Haines, H.; Matulewicz, M. C. *Biochem Pharmacol* **1994**, *47*, 2187-2192.

[108] Di Caro, A.; Perola, E.; Bartolini, B.; Marzano, M.; Liverani, L.; Mascellani, G.; Benedetto, A.; Cellai, L. *Antivir Chem Chemother* **1999**, *10*, 33-38.

[109] Smee, D. F.; Verbiscar, A. J. *Antiviral Chem Chemother* **1995**, *6*, 385-390.

[110] Balzarini, J.; Neyts, J.; Schols, D.; Hosoya, M.; Van Damme, E.; Peumans, W.; De Clercq, E. *Antiviral Res* **1992**, *18*, 191-207.

[111] Christensen, N. D.; Reed, C. A.; Culp, T. D.; Hermonat, P. L.; Howett, M. K.; Anderson, R. A. *Antimicrob Agents Chemother* **2001**, *45*, 3427-3432.

[112] Girond, S.; Crance, J. M.; Cuyck-Gandre, H.; Renaudet, J.; Deloince, R. *Res Virol* **1991**, *142*, 261-270.

[113] Takahashi, K.; Matsuda, M.; Ohashi, K.; Taniguchi, K.; Nakagomi, O.; Abe, Y.; Mori, M. *Antiviral Res* **2001**, *49*, 15-24.

[114] Serkedjieva, J.; Manolova, N.; Zgorniak, N., I; Zawilinska, B. *Phytother Res* **1990**, *4*, 97-100.

[115] Nagata, K.; Sakagami, H.; Harada, H.; Nonoyama, M.; Ishihama, A.; Konno, K. *Antiviral Res* **1990**, *13*, 11-21.

[116] Harada, H.; Sakagami, H.; Nagata, K.; Oh-hara, T.; Kawazoe, Y.; Ishihama, A.; Hata, N.; Misawa, Y.; Terada, H.; Konno, K. *Antiviral Res* **1991** , *15*, 41-49.

[117] Sakagami, H.; Takeda, M.; Kawazoe, Y.; Nagata, K.; Ishihama, A.; Ueda, M.; Yamazaki, S. *In Vivo* **1992**, *6*, 491-495.

[118] Sakagami, H.; Kawazoe, Y.; Komatsu, N.; Simpson, A.; Nonoyama, M.; Konno, K.; Yoshida, T.; Kuroiwa, Y.; Tanuma, S. *Anticancer Res* **1991**, *11*, 881-888.

[119] Hosoya, M.; Shigeta, S.; Ishii, T.; Suzuki, H.; De Clercq, E. *J Infect Dis* **1993**, *168*, 641-646.

[120] Hosoya, M.; Balzarini, J.; Shigeta, S.; De Clercq, E. *Antimicrob Agents Chemother* **1991**, *35*, 2515-2520.
[121] Ivanova, V.; Rouseva, R.; Kolarova, M.; Serkedjieva, J.; Rachev, R.; Manolova, N. *Prep Biochem* **1994**, *24*, 83-97.
[122] Tsybul'skii, A. V.; Ovodova, R. G.; Glazkova, V. E.; Besednova, N. N.; Ovodov, I.; Orlova, T. G.; Shcheglovitova, O. N.; Minskaia, L. A. *Vopr Virusol.* **1990**, *35*, 194-197.
[123] Tsybul'skii, A. V.; Isachkova, L. M.; Ovodova, R. G.; Glazkova, V. E.; Besednova, N. N.; Orlova, T. G. *Zh Mikrobiol Epidemiol Immunobiol* **1992**, *35*, 62-66.
[124] Ahmad, A. S.; Matsuda, M.; Shigeta, S.; Okutani, K. *Marine Biotechnol* **1999**, *1*, 102-106.
[125] Matsuda, M.; Shigeta, S.; Okutani, K. *Marine Biotechnol* **1999**, *1*, 68-73.
[126] Pietropaolo, V.; Seganti, L.; Marchetti, M.; Sinibaldi, L.; Orsi, N.; Nicoletti, R. *Res Virol* **1993**, *144*, 151-158.
[127] Mastromarino, P.; Petruzziello, R.; Macchia, S.; Rieti, S.; Nicoletti, R.; Orsi, N. *J Antimicrob Chemother* **1997**, *39*, 339-345.
[128] Garcia-Villalon, D.; Gil-Fernandez, C. *Antiviral Res* **1991**, *15*, 139-148.
[129] Crance, J. M.; Gratier, D.; Guimet, J.; Jouan, A. *Res Virol* **1997**, *148*, 353-365.
[130] Fabregas, J.; Garcia, D.; Fernandez, A. M.; Rocha, A., I; Gomez, P. P. *Antiviral Res* **1999**, *44*, 67-73.
[131] Smee, D. F.; Sidwell, R. W.; Huffman, J. H.; Huggins, J. W.; Kende, M.; Verbiscar, A. J. *Chemotherapy* **1996**, *42*, 286-293.
[132] Hudson, J. B.; Kim, J. H.; Lee, M. K.; DeWreede, R. E.; Hong, Y. K. *J Appl Phycol* **1998**, *10*, 427-434.
[133] Andrei, G.; De Clercq, E. *Antiviral Res* **1990**, *14*, 287-299.
[134] Gauntt, C. J.; Wood, H. J.; McDaniel, H. R.; McAnalley, B. H. *Phytother Res* **2000**, *14*, 261-266.
[135] Hotta, H.; Hagiwara, K.; Tabata, K.; Ito, W.; Homma, M. *Int J Immunopharmacol* **1993**, *15*, 55-60.
[136] Kovalenko, O. G.; Telegeeva, T. A.; Shtakun, A., V; Pogorila, Z. O. *Biopolim Kletka* **2000**, *16*, 53-59.

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# RECENT ADVANCES IN THE CHEMISTRY OF BIOACTIVE 3-DEOXY-ULOSONIC ACIDS

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**ABSTRACT:** This article describes recent developments in the chemistry of an important family of complex monosaccharides which have diverse structures and participate in a wide range of biological processes. For example: 3-deoxy-D-*manno*-2-octulosonic acid (KDO) is a key component of the lipopolysaccharides (LPS) of Gramm-negative bacteria, 3-deoxy-D-*arabino*-2-heptulosonic acid (DAH) is a key intermediate in the biosynthesis of aromatic amino acids in bacteria and plants. A number of their syntheses that were achieved by homologation reactions of the natural carbohydrate units using enzymatic or chemical methods, as well as by total synthetic approaches are here included. Special emphasis is placed on new methodologies and their correlation with the biosynthetic pathway of the corresponding ulosonic acids.

This chapter is not intended to cover all available literature, since other review articles have been published. It tries to demonstrate the real contribution, showing that the development of general and efficient strategies for the construction of these important compounds still remains as significant.

## INTRODUCTION

The 3-deoxy-2-ulosonic acids are a diverse family of natural carbohydrates, possessing a ketone functionalities at the α position of the carboxylic acids and 3-OH groups reduced. Another common feature is, that they belong to the "higher" sugars, as their molecules are usually composed of 7 to 9 carbon atoms [1]. Despite this, here belong also a few rare 3-deoxy-2-ulosonic acids (**1-3**) which have five or six-carbon atoms structure [2], as well as these bearing 3-OH group in the molecule, *i.e.* D-*lyxo*-hexulosonic acid **4** [3] and D-*glycero*-α-D-*talo*-octulosonic acid (KO, **8**) [4], isosteric to 3-deoxy-D-*manno*-octulosonic acid (**7**) [5] (Fig. 2).

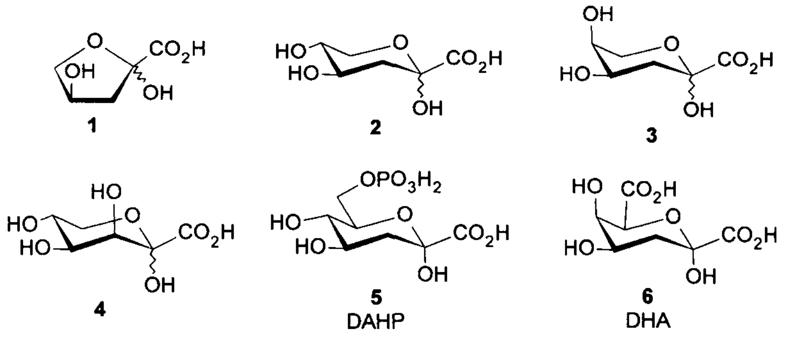



**Fig. (1).** Natural 3-deoxy-ulosonic acids

The diversity of these naturally occurring ulosonic acids concerns also the substituents present in their molecules. Thus, 3-deoxy-α-D-*arabino*-2-heptulosonic acid 7-phosphate (DAHP, **5**) possess a phosphate group at C-7 [6], 3-deoxy-α-D-*lyxo*-2-heptulosaric acid (DHA, **6**) [7-10] bears an additional carboxyl group at C-6 instead of the hydroxyl one, whereas *N*-acyl and *O*-acetyl derivatives of the isomeric 5,7-diamino-3,5,7,9-tetradeoxy-2-nonulosonic acids (pseudaminic and legionaminic acids, **12**) have two amino groups and C-9-deoxy function [11]. The most abundant in nature, *N*-acetylneuraminic acid **9** (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid) is able to incorporate to its molecule further acetyl groups, most commonly at C-9, but di- and tri-*O*-acetylated derivatives are also known. Lactoylation or phosphorylation may also occur at C-9, while C-8 hydroxyl can be methylated or sulfated [12]. Its amino function can be also glycoylated (Neu5Gc, **10**). Another compound, 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN, **11**) may be considered as a deaminated form of neuraminic acid [12]. In summary, 43 various naturally occurring sialic acid derivatives have been isolated [13].

The ulosonic acids participate in many important biological processes. 3-Deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (**5**) is a key intermediate in the biosynthesis of aromatic amino acids in plants and bacteria *via* shikimic acid pathway [6] and KDO (**7**) [1,5], DHA (**6**) [7-10] and KO (**8**) [4] are key components of the outer membrane of the lipopolysaccharides (LPS) of Gramm-negative bacteria, playing a crucial role in immunospecificity. Similarly act *N*-acyl and *O*-acetyl derivatives of the various isomeric pseudoaminic acids of type **12**, isolated from LPS

of the pathogenic bacteria *Pseudomonas aeruginosa*, *Shigella boydi*, *Legionella pneumophila* and others [11]. They are known as components of capsular polysaccharides [14], glycoproteins [15], and they are likely involved in bacterial virulence [16].

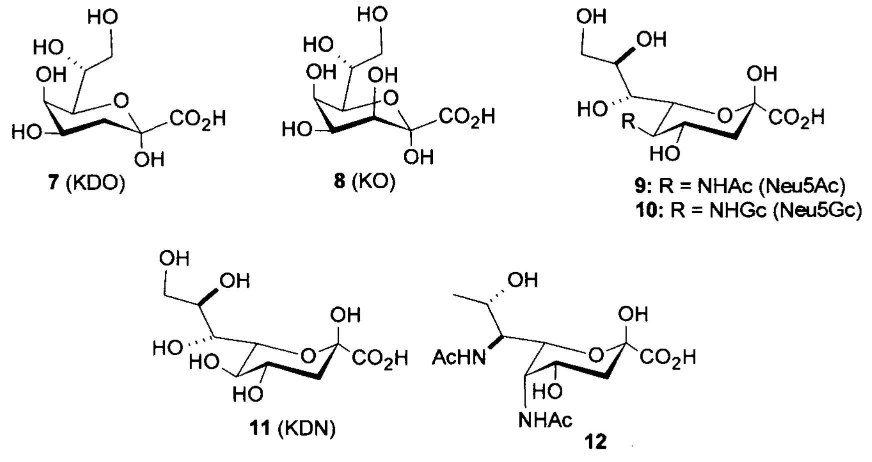



**Fig. (2).** Some natural octulo- and nonulosonic acids

An unique structure represents DHA (**6**), being a rare dicarboxylic (heptulosaric) acid [7]. It was isolated from the LPS of *Actinobacter calcoaceticus* [8] and other bacteria belonging to *Rhizobiaceae* [8]. Besides of bacteria, DHA has been so far observed only in plants, as a component of their rhamnogalacturononan [9] and as a cell-wall component of algae [10].

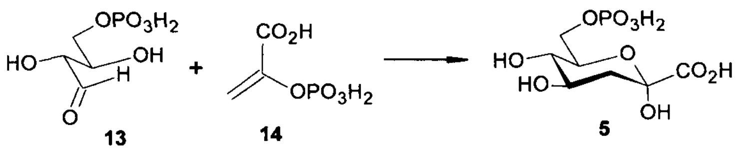
The eight carbons α-keto acid KDO (**7**), found in almost all Gramm-negative bacteria, links the polysaccharide part of LPS to the lipid A region [5]. It was also isolated from several extracellular polysaccharides.

Sialic acid (Neu5Ac, **9**) occurring mainly in various types of extracellular polysaccharides has also been observed in some LPS [12]. As the α-linked terminal substituent of the animal cell surface glycoproteins and glycolipids, it is ideally positioned to participate in carbohydrate-protein interactions, thus mediating in recognition phenomena [6]. These include acting as a receptor for bacteria and viruses, masking antigenic determinants, mediating the cell-cell adhesion and enabling the cell-cell communication processes [6,13,17].

*N*-Glycoyl substituted sialic acid Neu5Gc (**10**) is rather a rare sugar existing in echinoderms [18]. KDN (**11**) isolated originally from the membrane polysialoglycoproteins of rainbow trout eggs [19] has been more recently discovered in human fetal card red blood cells and cancer cells. It shows oncofetal antigen properties, important in the early detection of disease [20].

Such an important biological and immunological role of 3-deoxy ulosonic acids, as well as their unique chemical structure has emerged as an important area of many research fronts. Much attention has been also focused on enzymatic, chemoenzymatic, and stereocontrolled chemical syntheses of all of these compounds and their analogues.

Generally, syntheses of DAHP, KDO and Neu5Ac are based on chain extension of an appropriate sugar unit electrophiles by a $C_3$ nucleophiles, according to the biosynthetic pathway, shown in Scheme 1. Thus, the reaction of D-erythrose 4-phosphate (**13**) acting as a $C_4$ electrophile with phosphoenolpyruvate (**14**, $C_3$ nucleophile) creates DAHP [6]. Mutual relationship to this pathway concerns the biosynthesis of KDO and Neu5Ac, which are produced on the reaction of phosphoenolpyruvate with D-arabinose 5-phosphate ($C_5$ electrophile) or *N*-acetyl-D-mannosamine 6-phosphate ($C_6$ electrophile), respectively [5,12].




**Scheme (1).** Biosynthesis of DAHP

Recent contributions to the chemical syntheses of ulosonic acids are based also on another pathways, involving various types of the addition reactions of $C_1$, $C_2$ or more carbon atoms units to the sugars in an open-chain or pyranose form. Totally synthetic approaches have also been developed.

Assuming that the recent review articles concern mainly sialic acid (Neu5Ac) and its deaminated counterpart KDN, as well as their modified analogs [12], we found as not reasonable describing them in this review. Instead, more attention will be focused on the remaining 3-deoxy-2-ulosonic acids.

## ENZYMETIC SYNTHESES

Aldolase-catalyzed stereocontrolled aldol condensation is getting increasing acceptance in modern research and production as an alternative to classical chemical methodology [21-23]. The potential of enzymes as catalysts for the synthesis of carbohydrates has been also deeply explored [23-29].

Stereoselective formation of C-C bonds in nature is assisted by enzymes named lyases, which catalyze usually reversible addition of carbon nucleophiles to carbonyl group. Aldolases belong to the group of lyases. They occur in all organisms being involved in the metabolism of carbohydrates as well as amino- and hydroxy acids. Over 30 aldolases have been identified to date.

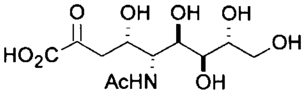
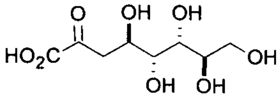
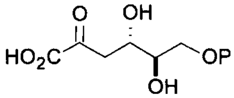
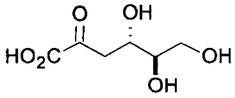
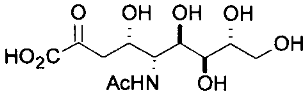
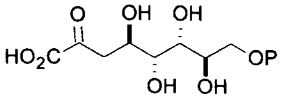
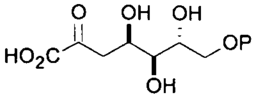
Although both types of aldolases are highly specific for the donor substrate they show some flexibility as far as the structure of the acceptor is concerned. Some of them accept variety of unnatural compounds. The stereoselectivity of the addition is in general controlled by the enzyme and does not depend on the structure of substrate. This rule is in force to almost all know aldolases with only few exceptions.

The aldolases which have been investigated for their synthetic utility can be classified on the basis of the donor substrate accepted by the enzyme. For the synthesis of 3-deoxy-2-ulosonic acids pyruvate- and phosphoenolpyruvate dependent aldolases are the most desirable enzymes as they are involved in the metabolism of sialic acids (or structurally related ones) *in vivo*. They use pyruvate or phosphoenolpyruvate as a donor to form 3-deoxy-2-keto acids (Table 1). Both of them add a three-carbons ketone fragment onto a carbonyl group of an aldehyde. The pyruvate dependent aldolases have a catabolic function *in vivo*, whereas the phosphoenolpyruvate dependent aldolases are involved in the biosynthesis of the keto acids. For synthetic purpose the equilibrium of the pyruvate dependent aldolases is shifted toward the condensation products through the use of an excess of pyruvate.

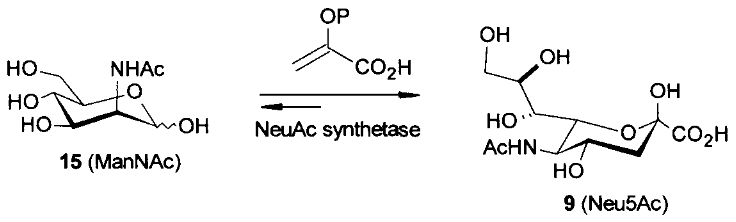
Since some excellent articles cover the enzymatic syntheses of sialic acids and their analogues [21,23,29], it is not authors intention to present them once again. The following chapter tries only to form an opinion on

catalytic usefulness of the enzymes for stereoselective synthesis of ulosonic acids.

**Table (1).** Some aldolases used for synthesis of 3-deoxy-2-ulosonic acids and their aldol products *in vivo*.

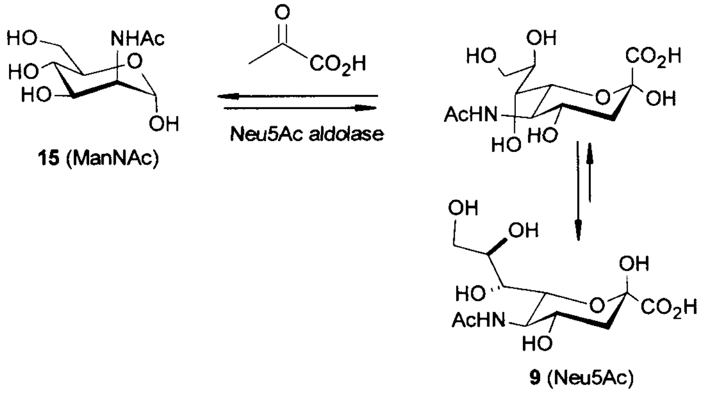
| Pyruvate-Dependent Aldolases | |
|---|---|
| *N*-Acetylneuraminic (Neu5Ac) aldolase EC 4.1.3.3 |  |
| 3-Deoxy-D-*manno*-octulosonate (KDO) aldolase EC 4.1.2.23 |  |
| 2-Keto-3-deoxy-6-phosphogluconate (KDPG) aldolase EC 4.1.2.14 |  |
| 2-Keto-3-deoxy-gluconate (KDG) aldolase extract from fungi *Aspargillus terrus* [67] |  |
| **Phosphoelnolpyruvate-Dependent Aldolases** | |
| *N*-Acetylneuraminic (Neu5Ac) synthetase EC 4.1.3.19 |  |
| 3-Deoxy-D-*manno*-octulosonate 8-phosphate (KDO 8-P) synthetase EC 4.1.2.16 |  |
| 3-Deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (DAHP) synthetase EC 4.1.2.15 |  |

Synthesis of *N*-acetylneuraminic acid (Neu5Ac) *in vivo* is catalyzed by Neu5Ac synthetase (EC 4.1.3.19) through the irreversible condensation of phosphoenolpyruvate (PEP) and *N*-acetylmannosamine (**15**) (Scheme 2) [30-32]. This enzyme has not yet been isolated and its catalytic activity might be interesting field for exploration.




**Scheme (2).** Biosynthesis of Neu5Ac from phosphoenolpyruvate and *N*-acetyl-D-mannosamine (ManNAc, **15**)

In contrast, Neu5Ac aldolase turned out to be very useful for synthetic purpose because it tolerate wide range of unnatural substrates [21,23]. The enzyme also named sialic acid aldolase (EC 4.1.3.3.) catalyze reversible reaction of *N*-acetyl-D-mannosamine (**15**) and pyruvate (Scheme 3) [33-35].



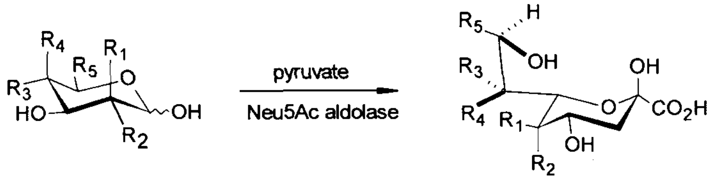

**Scheme (3).** Aldol reaction mediated *in vivo* by Neu5Ac aldolase

Neu5Ac aldolase has been isolated from many sources including bacteria and mammals. Significant interest toward sialic acid analogs and commercial availability of the aldolase, have led to the intensive studies on this enzyme, which resulted in the knowledge that despite its highly specificity for pyruvate [36] it accepts a number of different acceptor

substrates. Numerous five and six carbons aldoses have to date been demonstrated to be accepted by Neu5Ac aldolase [36-66].

Many hexoses were tested in both D- and L-form, resulting in the formation of nonulosonic acid analogues. Table 2 demonstrates the most common enzymatic approach to the synthesis of Neu5Ac (entry 1) and its analogues (entry 2-12) from modified *N*-acetylmannosamine derivatives.

**Table(2).** Neu5Ac analogs prepared using Neu5Ac aldolase.




| Entry | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Ref. |
|---|---|---|---|---|---|---|
| 1 | NHAc | H | OH | H | $CH_2OH$ | [36-48] |
| 2 | NHAc | H | OH | H | $CH_2OAc$ | [36,37,39,42] |
| 3 | NHAc | H | OH | H | $CH_2N_3$ | [42] |
| 4 | NHAc | H | OH | H | $CH_2F$ | [42] |
| 5 | NHAc | H | OH | H | $CH_2OR$ | [39,42] |
| 6 | NHAc | H | OMe | H | $CH_2OH$ | [39,49] |
| 7 | NHAc | H | $N_3$ | H | $CH_2OH$ | [50] |
| 8 | NHAc | H | H | H | $CH_2OH$ | [48,49] |
| 9 | NHCBz | H | OH | H | $CH_2OH$ | [44,51] |
| 10 | NHCOR | H | OH | H | $CH_2OH$ | [39,52] |
| 11 | NHCSMe | H | OH | H | $CH_2OH$ | [53] |
| 12 | $N_3$ | H | OH | H | $CH_2OH$ | [41,54,55] |
| 13 | OH | H | OH | H | $CH_2OH$ | [42,45,48,49,56] |
| 14 | OH | H | OH | H | H | [41,48,56] |
| 15 | OH | H | H | H | $CH_2OH$ | [41,48] |
| 16 | OH | H | OH | F | $CH_2F$ | [42] |
| 17 | H | H | OH | H | $CH_2OH$ | [41] |
| 18 | H | H | H | OH | H | [41] |
| 19 | H | H | OH | H | $CH_2OH$ | [41,42,48,56] |
| 20 | H | F | OH | H | $CH_2OH$ | [42] |
| 21 | Ph | H | OH | H | $CH_2OH$ | [48] |

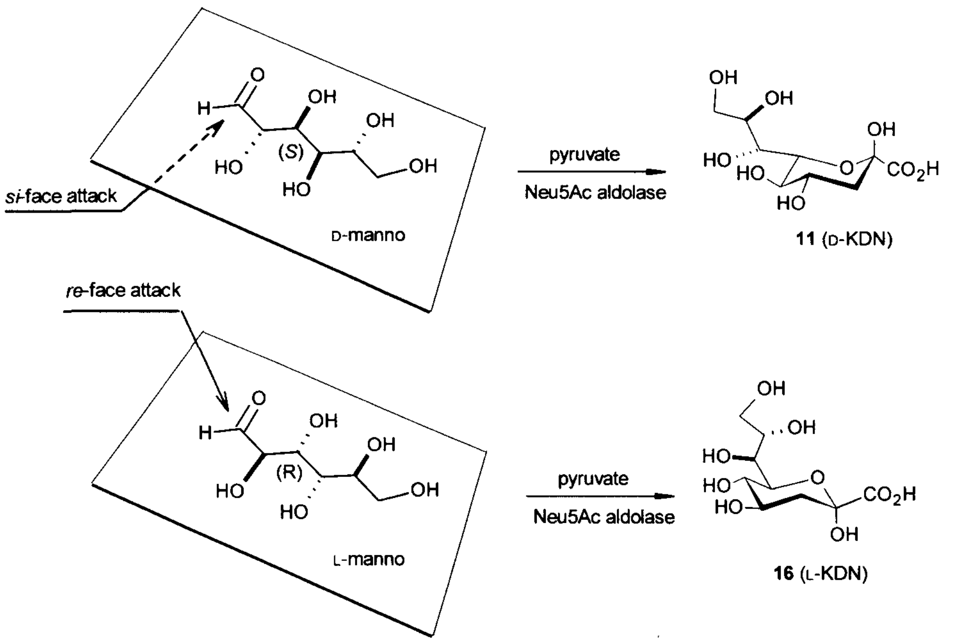
This approach offers a convenient and, in general, an efficient large scale production method [38,40,46].

Neu5CBz (entry 9) and 5-azido-5-deacetamido-Neu (entry 12) have been prepared by treatment of the corresponding 2-modified mannose derivatives with pyruvate in the presence of Neu5Ac aldolase. The C-5 azido group may be easily converted to the free amine. Synthesis of 7-amino-substituted Neu5Ac derivative has been presented [50] *via* azido derivative as an alternative route to the particularly difficult chemical

synthesis of this class of compounds. α-Methyl ketoside of the *N*-unprotected Neu5Ac was proved to be a substrate in the preparation of *N*-substituted NeuAc derivatives [57]. Synthesis of *N*-glycoylneuraminic acid derivatives found in some glycolypids and glycoproteins of certain species of animals has been presented as well [52]. Although some C-3 nitrogen functionalized ManNAc derivatives were prepared, none of them was found to be the substrate for Neu5Ac aldolase [61].

Some deaminated Neu5Ac analogues were also prepared (rows 13-21), including 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN, entry 13).

Free hydroxyl group at C-3 is a condition *sine qua non* for aldol condensation [36,58]. Substitution at C-2 of *N*-acetyl mannosamine is tolerated, and the enzyme exhibits only a slight preference for the defined stereochemistry at the other centers.



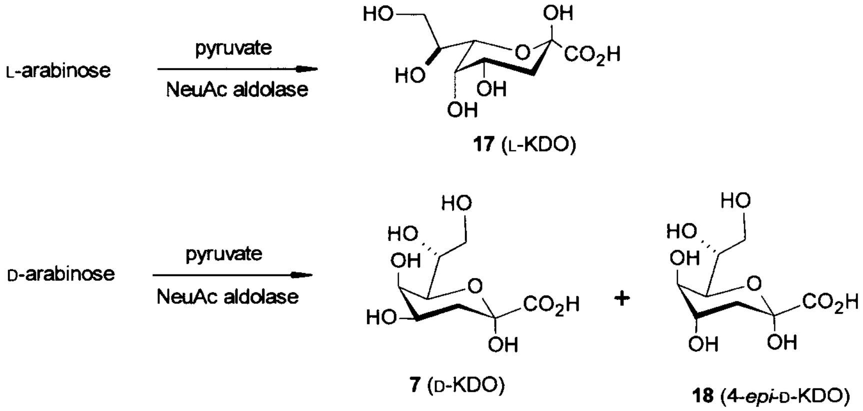

**Scheme (4).** Neu5Ac aldolase catalyzed reaction of *N*-acetyl-D- and L-mannosamine

Unlike the most known aldolases the stereochemical outcome of reactions catalyzed by Neu5Ac aldolase depends on the structure of the substrate. In substrates with the natural *S* configuration at C-3

(*e.g.* ManNAc), the carbonyl group is attacked from the *si*-face to form a new stereogenic center with *S* configuration leading to so-called KDN-type product (Scheme 4) [45,47,48]. With some substrates, however (*e.g.* L-mannose), the stereochemical course of the reaction can be reversed: the enamine formed in the active site attacks the carbonyl component from the *re*-face, and the C-4 center of the resulting product has the *R* configuration (so-called KDO-type) [43]. This observation was utilized in the syntheses of unnatural sialic acid analogs like L-Neu5Ac, or L-KDN presented by Wong from L-mannosamine, and *N*-acetyl-L-mannose [43,45].

Some observations indicate that the stereochemical outcome of the reaction seems to be under thermodynamic control, and stereochemistry at C-4 can be reversed. For example, in the Neu5Ac aldolase catalyzed synthesis of KDO, a mixture of (*S*)-C-4 and (*R*)-C-4 were isolated when D-arabinose was the substrate (Scheme 5) [47,48].

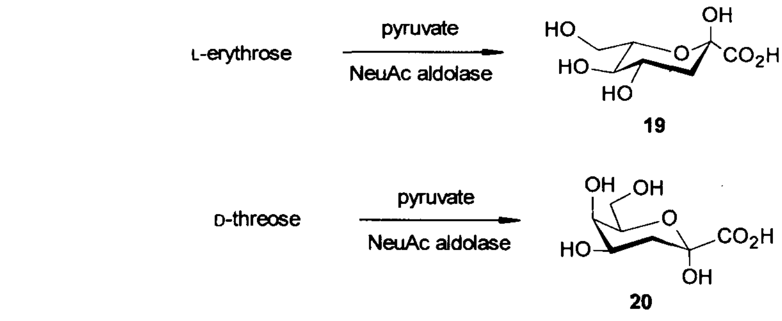
For further study on this problem see ref. 23, 43, 45, 47 and 58.




**Scheme (5).** Some unnatural sialic acid analogues synthesized with Neu5Ac aldolase

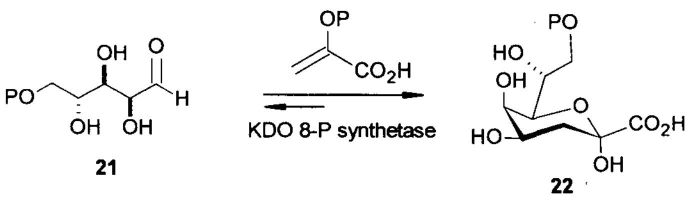
Some tetroses are also substrates for the enzyme [58]. The reaction of L-erythrose led to heptulosonic acid **19**, while D-isomer gave DHA precursor **20** (accompanied by the α/β–furanose) (Scheme 6).

A sequential aldol reaction leading to sialic acid derivatives has been also presented [59]. Combination of acetaldehyde dependent aldolase (DERA) and Neu5Ac aldolase catalyzed reactions led to (*R*)-C-4 keto acids. In this case, however, one-pot reaction sequence was not possible due to the incompatibility of the reaction conditions for two enzymes.



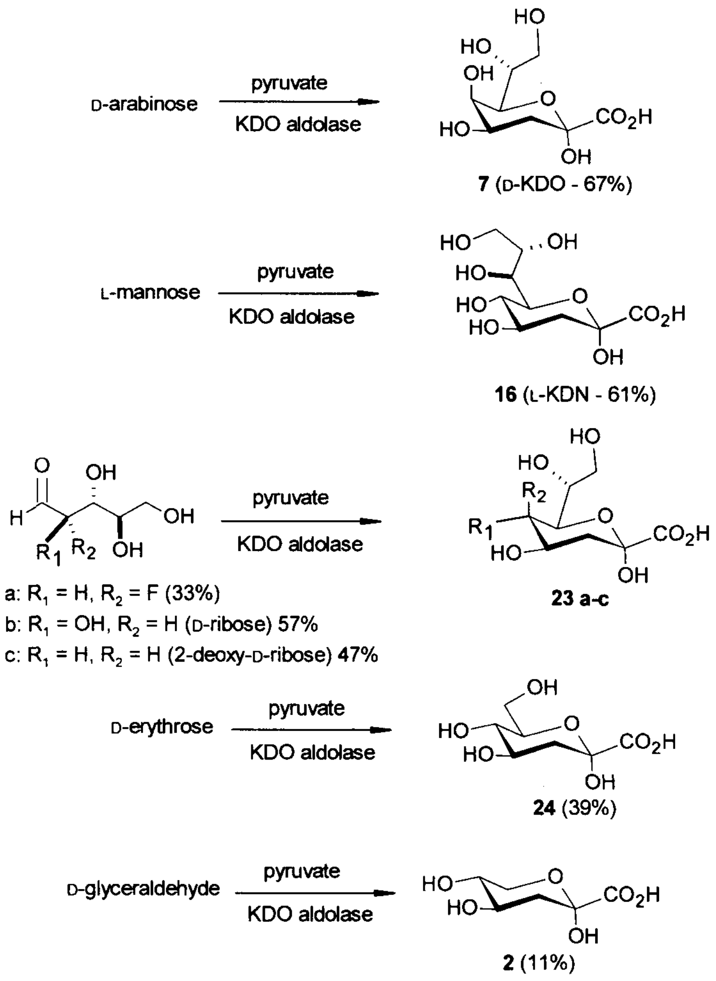

**Scheme (6).** Condensation of tetroses catalyzed by Neu5Ac aldolase

In the biosynthesis of bacterial lipopolysaccharides 3-deoxy-D-*manno*-2-octulosonate 8-phosphate (KDO 8-P) synthetase (EC 4.1.2.16) is involved in catalyzing the irreversible aldol reaction of phosphoenolpyruvate (PEP) and D-arabinose 5-phospahate (**21**) to give KDO 8-P (**22**) (Scheme 7) [62]. The aldolase is highly specific for its natural substrates [63]. Preparative scale synthesis of KDO 8-P has been reported from D-arabinose (Ara) and phosphoenolpyruvate (PEP). Production of D-Ara 5-P from Ara using another enzyme - hexokinase - is the key step in the synthesis [63a].




**Scheme (7).** Biosynthesis of KDO 8-P

3-Deoxy-D-*manno*-2-octulosonate aldolase (EC 4.1.2.23), also named 2-keto-3-deoxyoctonate (KDO) aldolase catalyzes reversible condensation of pyruvate with D-arabinose to form KDO. Preliminary investigations of the substrate specificity indicated a high specificity for KDO in the direction of the cleavage. KDO aldolase has been tested in the condensation reaction with several unnatural substrates including D-ribose, D-xylose, D-lyxose, L-arabinose, D-arabinose 5-phosphate and *N*-acetylmannosamine, giving poor yield in all cases [64].

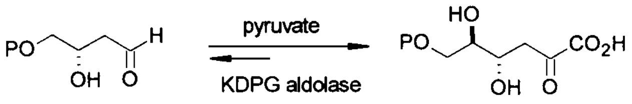



**Scheme (8).** KDO and its analogs synthesised by KDO aldolase

More recently Wong reported the study on a KDO aldolase isolated from *Auereobacterium barkerei*. The enzyme has been proved as a versatile catalyst in the synthesis of KDO and its analogues [65] (Scheme 8). This enzyme accepts hexoses, pentoses, tetroses and even trioses as substrates. Various aldol reactions were conducted on a preparative scale with significant success. KDO (7) was obtained from D-arabinose in 67% yield [65]. The enzyme was found to be specific for substrates having *R*-configuration at C-3, whereas the stereochemical requirements at C-2 were less important. In all cases observed so far,

pyruvate attacked the aldolase substrate stereoselectively on the *re*-face of the carbonyl group [65].

Some initial studies have been made on KDPG aldolase (EC 4.1.2.14) which *in vivo* catalyzes the reversible condensation of the pyruvate with glyceraldehyde 3-phosphate (**25**) to form 2-keto-3-deoxy-6-phosphogluconate (KDPG - **26**) (Scheme 9). The enzyme was found to accept a number of unnatural aldehydes as electrophiles. It was applied for the synthesis of KDG from glyceraldehyde [66].

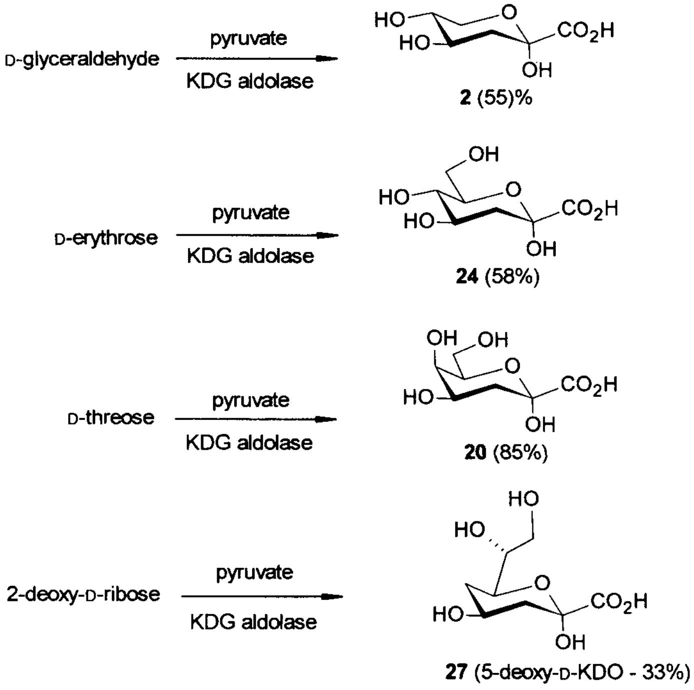


**25** **26**

**Scheme (9).** KDPG aldolase action *in vivo*

3-Deoxy-2-keto-gluconate (KDG) aldolase has been found in some filamentous fungi (e.g. *Aspergillus niger*) [67]. Its metabolic function is the degradation of gluconate *via* 2-keto-3-deoxy-D-gluconate into D-glyceraldehyde and pyruvate. Extract from *Aspergillus niger* did not catalyze a totally diastereoselective aldol reaction between pyruvate and D-glyceraldehyde *in vitro* [67a]. In contrast, extract from *Aspergillus terrus* turned out to be more efficient [67b]. Pure 2-keto-3-deoxy-ulosonic acids: KDG (**2**), DAH (**24**), and its C-5-epimer (**20**) were prepared on preparative scale through the diastereoselective aldol reaction of the pyruvate with D-glyceraldehyde, D-erythrose and D-threose, respectively. From 2-deoxy-D-ribose 5-deoxy-KDO (**27**) was obtained in a lower yield, with a diastereomeric excess of 72% (Scheme 10) [67b].

The 3-deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (DAHP) synthetase (EC 4.1.2.15) is an enzyme involved in the shikimic pathway of aromatic amino acids biosynthesis in bacteria and plants, where catalyzes the construction of 3-deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate from phosphoenolpyruvate and D-erythrose 4-phosphate [6]. Although 3-deoxy-D-*arabino*-2-heptulosonic acid 7-phosphate (DAHP) synthetase has not been widely investigated it has been employed for the DAHP synthesis on preparative scale from D-fructose in multienzyme system [68]. This one-pot synthesis was subsequently even more simplified by the results of further studies which indicated that it was more efficient and economical to use the whole cells containing a DAHP synthetase plasmid [69].




**Scheme (10).** Syntheses designed with KDG aldolase from *Aspergillus niger*

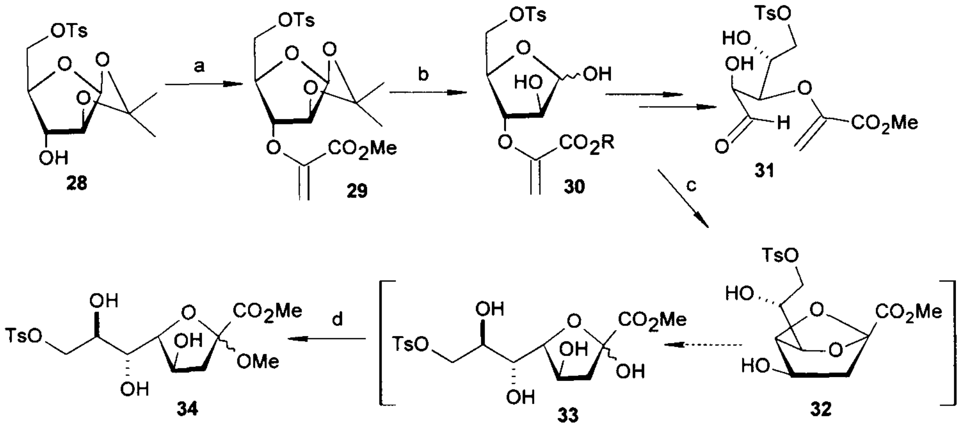
## CHEMICAL SYNTHESES

### Intramolecular Aldol Condensations

Development of the chemical synthesis of ulosonic acids which mimics the enzymatic condensation is an interesting target [70]. As a model a reaction leading to KDO 8-phosphate (**22**) has been evaluated. In this reaction mediated by KDO 8-P synthetase D-arabinose 5-phosphate (A5P, **21**) is reacted with phosphoenolpyruvate (PEP) (Scheme 7) [71].

Using this model and relying on the possible relationship between enzyme and intramolecular catalyzis [72] the novel, stereospecific synthesis of KDO has been developed [73]. The key step of the synthesis was an introduction of the enolpyruvate group to 3-OH in the arabinose derivative **28**, which was accomplished in four steps depicted in Scheme 11. On treatment of the enolpyruvate **30** with $SnCl_4$ in dichloromethane,

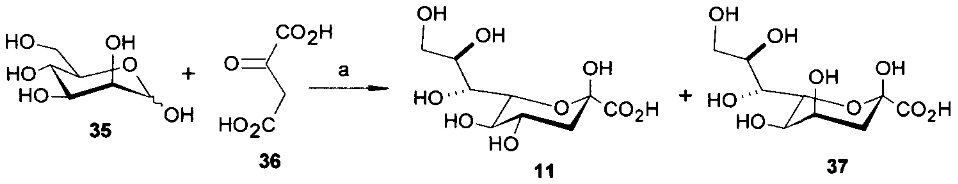
the bicyclic compound **32** was formed, which underwent the opening reaction of the ketal ring, to afford the anomeric mixture of KDO derivatives **34**. The synthesis is fully stereospecific and represents the first example of non-enzymatic addition of the enolpyruvate moiety to the carbonyl group in an intramolecular process.



**Scheme (11).** Reagents: (a) 1. $N_2C(CO_2Me)_2$, $Rh(OAc)_4$, 2. $[CH_2=NMe_2]^+I^-$, TEA 3. MeI, 4. DMSO, (b) AcOH, $H_2O$, (c) $SnCl_4$, (d) MeOH, PTSA.

## Aldol Condensations

The introduction of the α-keto acid function on the way to the ulosonic acids is a main problem of their syntheses. By analogy with the biosynthetic pathway, the aldol reaction between sugar aldehydes and a pyruvate equivalents seems to be the most simple and versatile. As it has been demonstrated by Cornforth [74] in the first chemical synthesis, the reaction of arabinose and oxalacetic acid as pyruvate equivalent, followed by decarboxylation afforded KDO, albeit in low yield. This condensation has been optimized by use of Ni(II) catalyst for the decarboxylation [75]. In this case, reaction of D-mannose and oxalacetic acid gave KDN (**11**) and its D-*manno* epimer **37** in 70% yield [75] (Scheme 12).




Scheme (12). Reagents: (a) 1.OH⁻, 2. $NiCl_2$, **11**/**37** = (4.2:1).

However, the lack of stereochemical control at C-4 of newly created asymmetric center, resulting in the formation of two diastereomers, is the great disadvantage. Despite this, Cornforth's methodology remains still the best choice for preparation of the selected ulosonic acids. It is a case of synthesis of nine stereoisomeric 5,7-diacetamido-3,5,7,9-tetradeoxy-2-nonulosonic acids [76]. The synthesis was performed by condensation of an appropriate 2,4-diacetamido-2,4,6-trideoxy-hexopiranoses with oxalacetic acid under basic conditions (Scheme 13), used previously in the preparation of Neu5Ac [77].

In all cases but one, two isomers were isolated in ~ 1:1 ratio, in low yield. Condensation of L-*allo* isomer **41** resulted in the formation of three isomers (**48**, **12** and **49**), evidently due to epimerisation at C-2 in the starting sugar prior to condensation (Scheme 13).

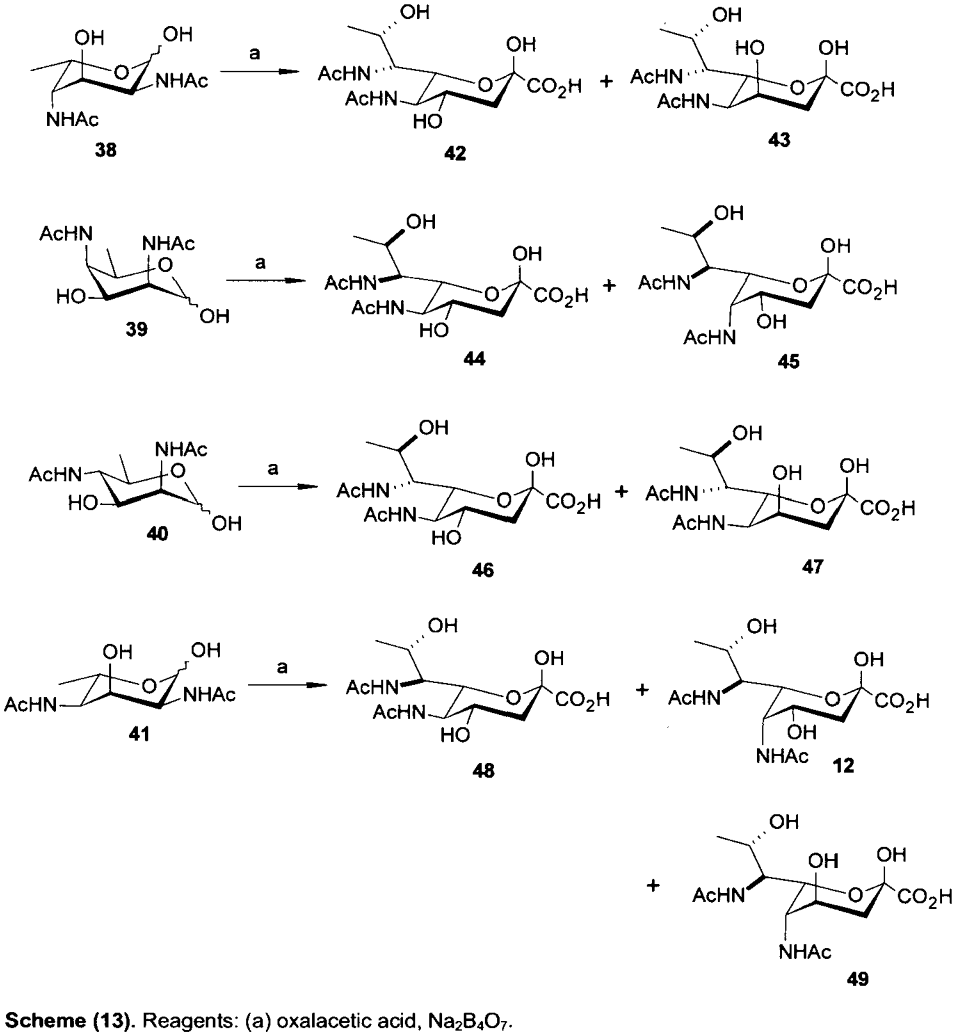
## Aldol Syntheses from Acyclic Aldoses

Much effort towards stereoselective synthesis of 3-deoxy-2-ulosonic acids resulted in the development of several strategies based on the diverse building units. Among them aldol reaction between D-arabinose derivative **50** and methyl dichloroacetate (**51**) deserves some attention [78] (Scheme 14). The condensation product isolated as oxirane derivative **52** reacted smoothly with $MgI_2$ to give the intermediate **53**, easily convertible into 3-deoxy-D-*arabino*-2-heptulosonic acid methyl ester **56** in high yield.

This strategy was also used in the stereoselective synthesis of L-*xylo*-heptulosonic acid [79] as well as D-*manno*-octulosonic acid (KDO) [80]. It is worth to note that the mannose derivative when used in hemiacetal form, failed to react with methyl dihaloacetate [80b].

One carbon atom elongation procedure has been developed by Knowles [81]. It was performed *via* acylation of 1,3-dithianyl derivative

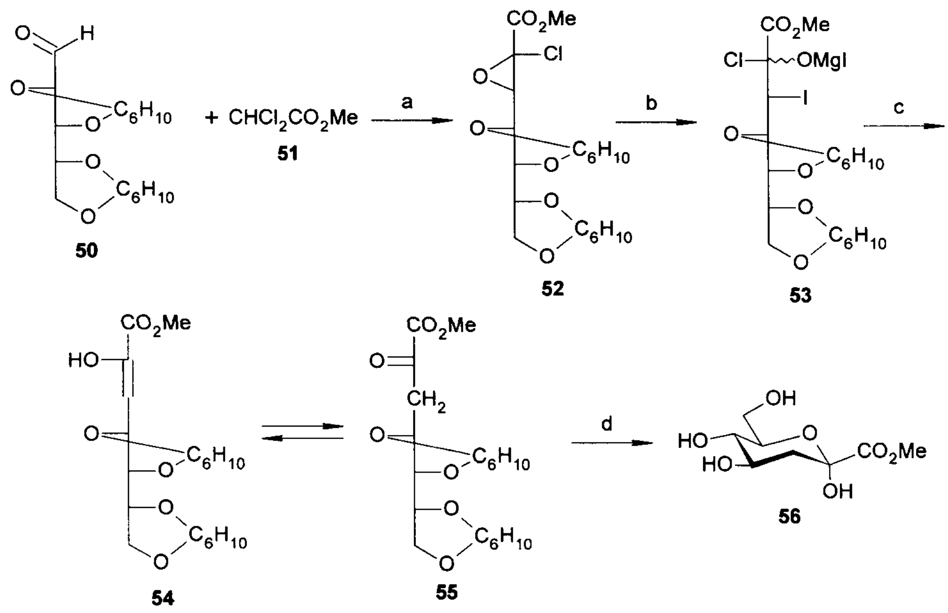
of 2-deoxy-D-glucose **57** with methyl chloroformate, followed by oxidative hydrolysis of the dithianyl residue (Scheme 15). Removal of the protected groups under acidic condition proceeded with concomitant cyclization, to afford DAH derivative **56** in 55% yield.



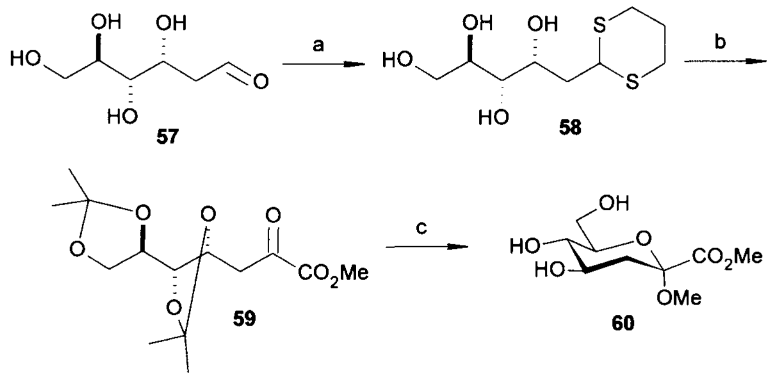

**Scheme (13).** Reagents: (a) oxalacetic acid, $Na_2B_4O_7$.

A more general approach to the synthesis of 3-deoxy-ulosonic acids has been presented by Dondoni [82]. The method consisted in the *anti*-aldol condensation of acyclic aldoses of type **61** with the lithiated 2-acetylthiazole as an equivalent of pyruvic acid to give **62** (Scheme 16).

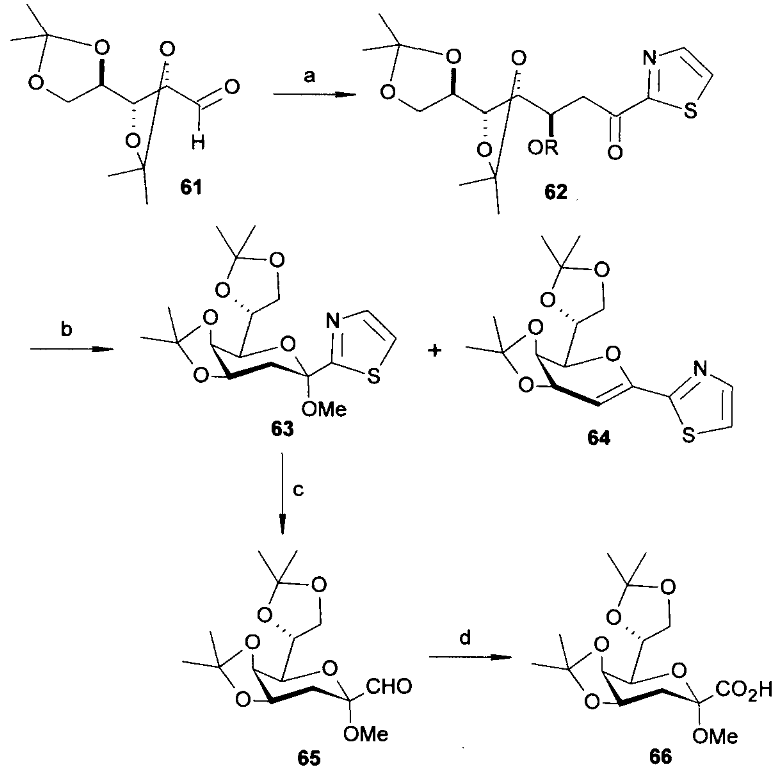
Hydrolytic cleavage of thiazole ring led to the corresponding precursor **65** in which aldehyde function has been oxidized to the desired carboxylic one, as it is shown in Scheme 16 demonstrating the synthesis of KDO.



**Scheme (14).** Reagents: (a) NaH, (b) $MgI_2$, (c) $NaHSO_3$, $H_2O$, (d) TFAaq.



**Scheme (15).** Reagents: (a) $HS(CH_2)_3SH$, HCl, (b) 1. acetone, $H^+$, 2. n-BuLi, $ClCO_2Me$, 3. NBS, acetone, (c) HBr, MeOH.



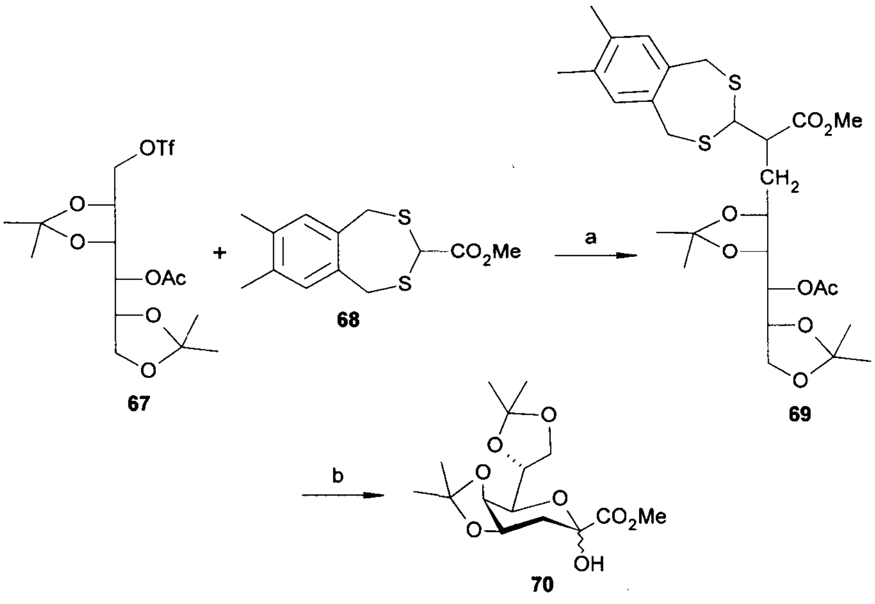

**Scheme (16).** Reagents: (a) 2-acetylthiazole, *t*-BuOH, BuLi, (b) 1. MeOH, HCl, 2. DMP, PTSA, acetone, **63**/**64** = (5:1), (c) 1. MeI, 2. $NaBH_4$, MeOH, 3. $HgCl_2$, (d) $Ag_2O$.

The above presented condensation reaction has been reported as highly diastereoselective, to afford the *anti* isomer **62** in ~ 90% yield. This was also true for the reaction of the other aldehydo sugars with the lithiated 2-acetylthiazole [82b], all furnishing *anti* products in 82-92% ee. Such an outcome of these reactions has been explained by the Felkin-Ahn-Houk model for asymmetric induction of reactions, proceeding *via* a non chelate-controlled process [82b,83]. Using this reaction sequence 3-deoxy-D-*erythro*-hexulosonic acid, as well as 3-deoxy-D-*ribo*-heptulosonic acid were prepared [82b]. However, this methodology suffers from some inconveniences, *i.e. i)* the condensation process proceeded under very mild conditions, otherwise a destruction of the thiazole moiety took place, *ii)* hemiacetalization reaction of the product **62** is accompanied by an elimination process, furnishing ~ 65% of desired

63 along with the by-product 64 (11%), *iii)* the "unmasking" procedure of the carboxyl group by hydrolytic cleavage of the thiazole unit, *via* formyl intermediate, seems to be rather tedious resulting in low yield.

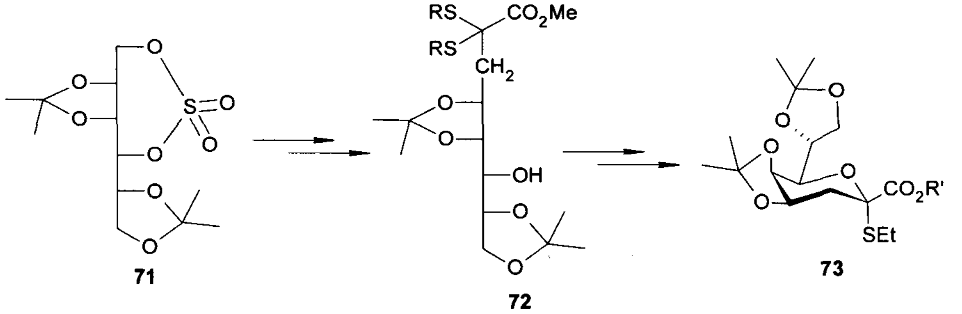
## Aldol Reactions from Alditols

Among various strategies towards ulosonic acids various acylation reactions are often used. They involve alditol derivatives as convenient substrates, and diverse acyl anion equivalents. Here belongs the synthesis of KDO, developed by Shiba *et al.* [84]. By reaction of the triflate **67** with the lithiated **68**, eight carbon atoms compound **69** was obtained (Scheme 17). Removal of *O*-acetyl residue with subsequent NBS promoted oxidative hydrolysis of the dithioacetal moiety resulted in the formation of KDO derivative **70**.




**Scheme (17).** Reagents: (a) BuLi, (b) 1. MeONa, 2. NBSaq.

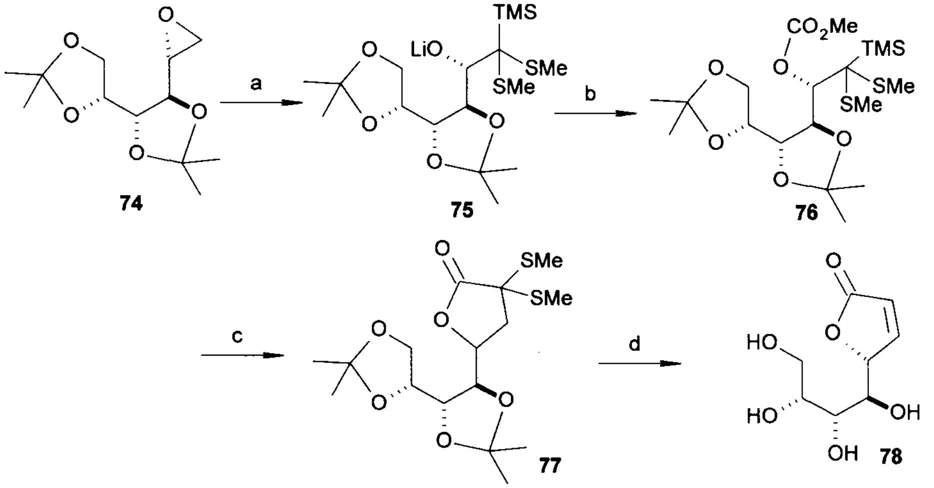
Applying analogous substrates and reaction sequence, KDO derivative **73** was produced in the form of thioglycoside (Scheme 18) [85].




**Scheme (18).**

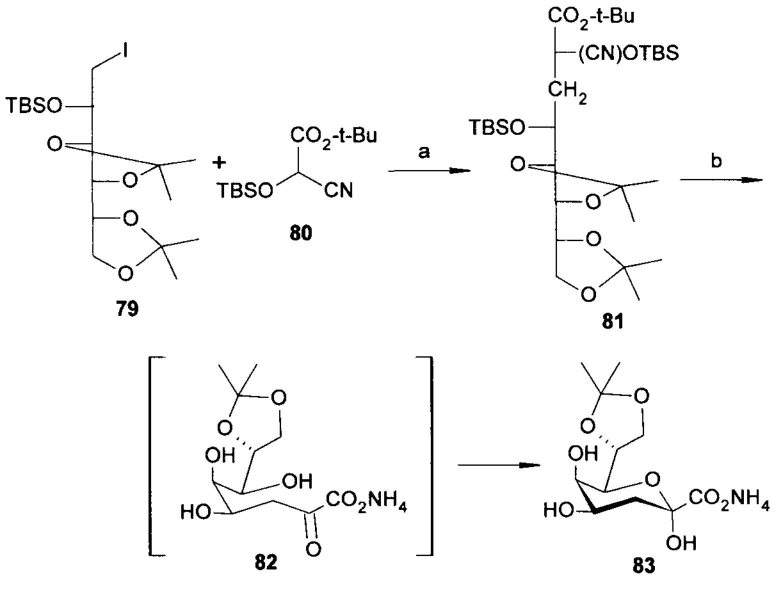
Similarly, 3-deoxy-α-D-*arabino*-2-heptulosonic acid (DHA) was prepared, starting from 1,4-cyclic sulfate and D-arabinose [86].

More recently, a strategy based on the reaction of 1,2-anhydro-3,4:5,6-di-*O*-isopropylidene-D-mannitol (**74**) with the lithiated silyl dithioacetal has been reported [87]. As a result, the alcohol **75** was formed which under acylation with methyl chloroformate furnished carbonate **76** (Scheme 19), converted further to the lactone **77**. Direct transformation of dithioacetal **77** into the corresponding ketone failed, despite the different methods used [88]. It succeeded in two steps procedure, involving its conversion into dimethylketal, followed by hydrolysis [89], to afford **78**, being the known precursor of KDO [90].



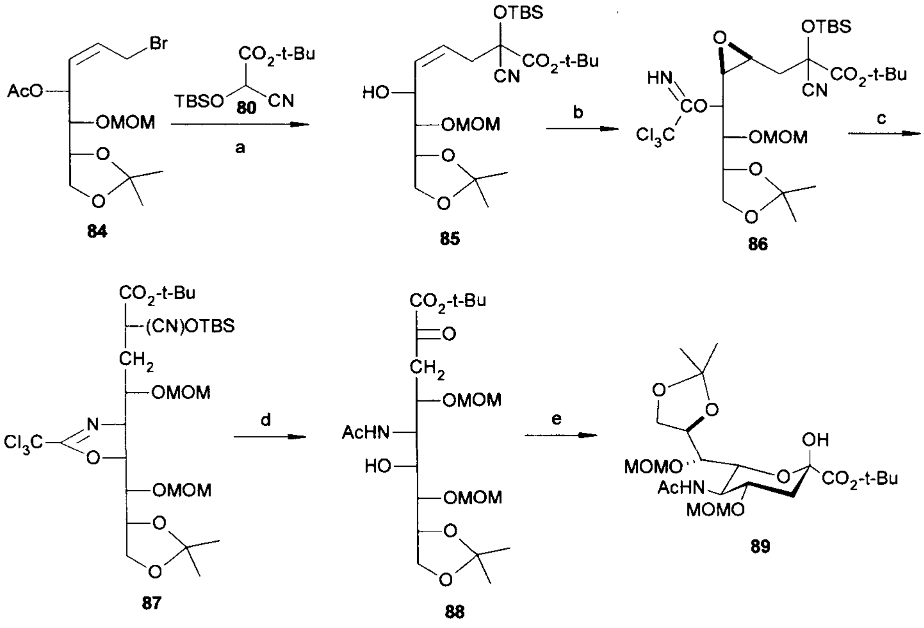

**Scheme (19).** Reagents: (a) tris(methylthio)methane, BuLi, (b) $ClCO_2Me$, (c) TBAF, (d) 1. [bis(trifluoroacetoxy)iodo]benzene, MeOH, 2. TFAaq.

An interesting approach towards KDO, KDN and Neu5Ac as well, utilized a protected cyanohydrine **80** as a versatile acylanion equivalent of alkyl glyoxylate. Its reaction with appropriate halogeno alditol, as it is depicted in Scheme 20, provided the alkylated product **81** in good yield [91]. Conversion of **81** into desired KDO derivative **83** was achieved *via* α-ketoacid **82**, resulted from hydrolysis of cyano group with $NH_3$aq.




**Scheme (20).** Reagents: (a) NaH, EtOH, (b) 1. TFAaq, MeOH, 2. $NH_3$aq.

In an extension of the previous work [91] Takahashi and co-workers utilized the above procedure for the preparation of KDN and Neu5Ac [92]. In both these syntheses a sugar derived heptosyl bromide **84** was taken as a substrate (Scheme 21). It was reacted with cyanoacetate leading to *Z*-olefin **85**. Stereo- and regioselective functionalization of the double bond in alcohol **85** *via* oxirane intermediate, and the introduction of acetamido group through trichloroacetimidate furnished **87**, transformed into neuraminic acid derivative according to the previously described procedure.



**Scheme (21).** Reagents: (a) 1. NaH, 2. $K_2CO_3$, MeOH, (b) 1. MCPBA, $Na_2HPO_4 \cdot 2H_2O$, 2. $Cl_3CCN$, DBU, (c) 1. $BF_3 \cdot OEt_2$, 2. MOMCl, $i$-$Pr_2EtN$, (d) 1. PTSA, 2. $Bu_3SnH$, AIBN, (e) 1. $Bu_4NF$.

## Syntheses *via* Wittig Reaction

Numerous syntheses of 3-deoxy-ulosonic acids are based on the Wittig reaction and its modifications. For this purpose various Wittig-type ylides, shown in Fig.(3), were examined.

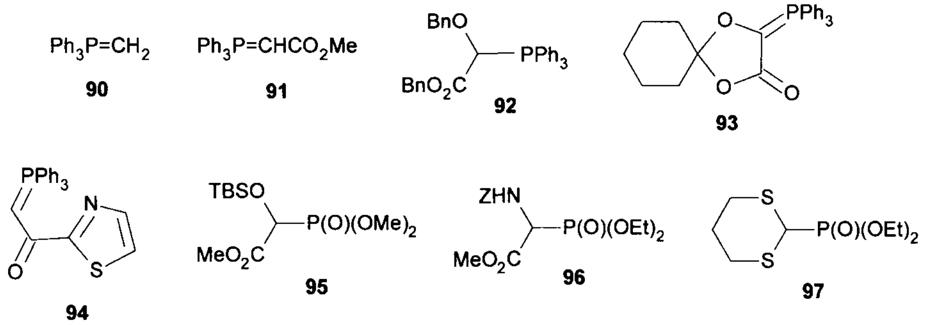
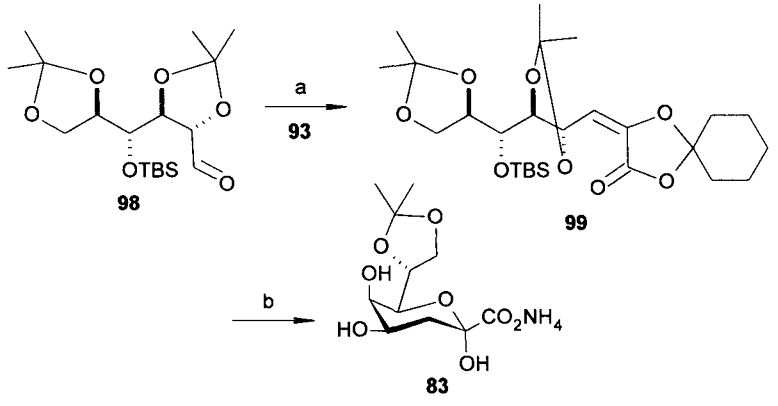


**Fig. (3).** Various Wittig ylide applied for the ulosonic acids synthesis.

In an elegant synthesis of KDG (3-deoxy-D-glucoronic acid), DAH and KDO, the reagent **93** was shown as a very useful [93]. The synthesis proceeded through 5-ylidene-1,4-dioxalan-4-one intermediate. In the case of KDO it was formed from the protected mannose-derived aldehyde **98** and ylide **93**, generated from its phosphonium bromide (Scheme 22).



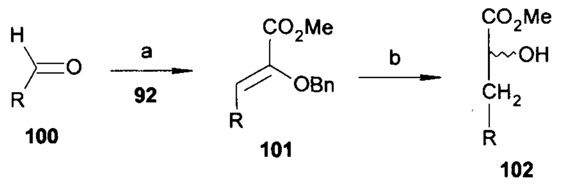

**Scheme (22).** Reagents: (a) benzene, (b) 1. AcOHaq, 2. $Ba(OH)_2$, 3. $(NH_4)_2SO_4$.

In order to transform the *E*/*Z* olefin (2:1) **99**, obtained in 74% yield, into KDO, the acid-labile protecting groups were removed by hydrolysis with aq. AcOH at 90 °C. The deprotected product, when treated with $Ba(OH)_2$, underwent an intramolecular cyclization to give KDO-Ba(II) salt, easily converted with $(NH_4)_2SO_4$ into the ammonium salt **83**.

The parallel, more convenient synthesis of KDO has been proposed by Schmidt and co-workers [94]. Employing the ylide **92** and aldehyde **98** (protected with Bn group instead of TBS), the corresponding olefin was produced in 73% yield. Its transformation was simply achieved by hydrogenolytic debenzylation, proceeding with concomitant intramolecular cyclization, to furnish after treatment with diazomethane, the anomeric mixture α/β (2:1) of KDO methyl ester.

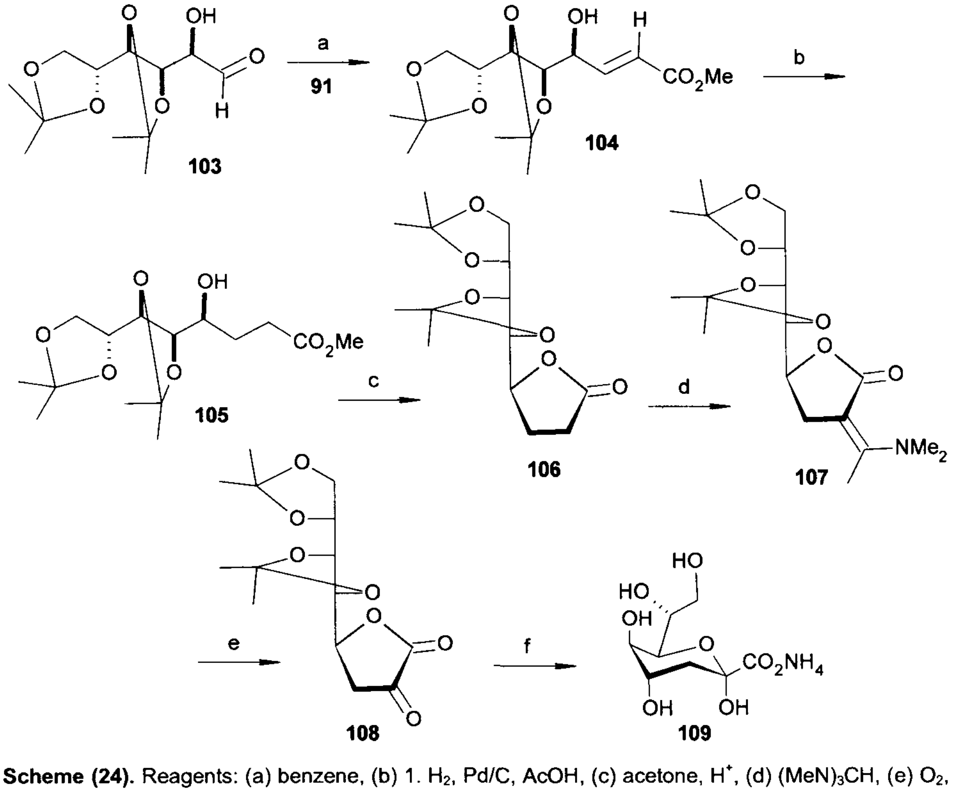
Since the reaction with the [(benzyloxy)(benzyloxycarbonyl)methyl] trimethylphosphonium ylide **92** was quite successful, it was also used to couple the isomeric D-*altro*-, D-*ido*-, D-*talo*-, and D-*gulo*-per-*O*-benzylated aldehydes [94b]. Transesterification of the products with methanol gave exclusively *Z*-isomers in all of the cases. Debenzylation enabled the selective cleavage of the enol ether functions. Subsequent

reduction of the liberated keto groups led to the stable derivatives, which were obtained as diastereomeric mixture of alcohols **102** of D-*glycero*-D-*gluco*/*manno*, D-*glycero*-D-*allo*/*altro* and D-*glycero*-D-*galacto*/*talo* configuration.




**Scheme (23).** Reagents: (a) 1. BuLi, 2. NaOMe, MeOH, (b) 1. $H_2$, Pd/C/$CaCO_3$, 2. $NaBH_4$.

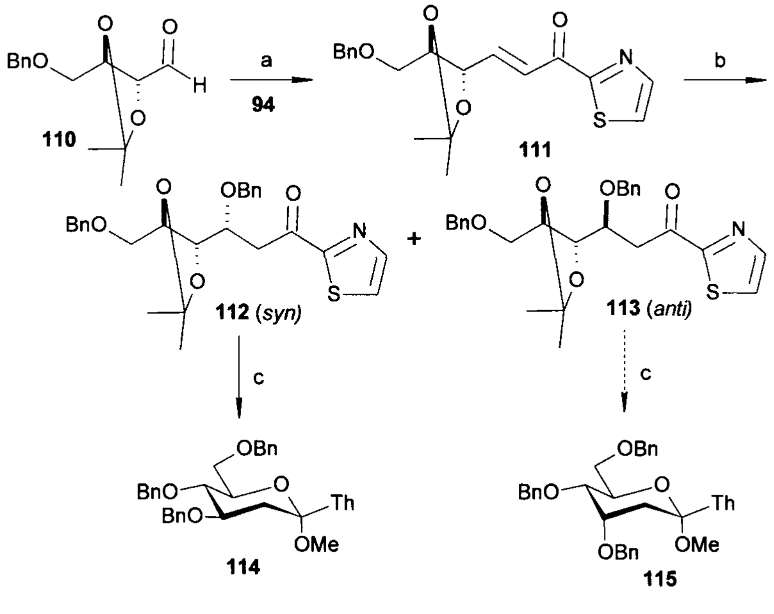
Following the route *via* Wittig reaction, a procedure leading to the D-*gluco*-KDO (3-deoxy-D-*gluco*-2-octulosonic acid) has been elaborated [95] (Scheme 24).




**Scheme (24).** Reagents: (a) benzene, (b) 1. $H_2$, Pd/C, AcOH, (c) acetone, $H^+$, (d) $(MeN)_3CH$, (e) $O_2$, hν, (f) 1. TFAaq, 2. $NH_3$aq.

Wittig olefin **104** formed in a reaction of the ylide **91** and a protected D-glucose aldehyde **103** was transformed to the lactone **106** under hydrogenation and subsequent cyclization. In the next step an oxo-function at the α position was introduced *via* *exo*-enamine system, followed by photooxidation. Final deprotection of **108**, then treatment with ammonia afforded D-*gluco*-KDO ammonium salt **109**.

An alternative ylide used in the Wittig reactions leading to 3-deoxy-2-ulosonic acids has been reported by Dondoni and co-workers [96]. This ylide (**94**, 2-TCMP) was generated by sequential reaction of 2-bromothiazole with bromomethylethoxylate, triphenylphosphine and sodium hydroxide. The Wittig olefination of sugar aldehydes with the 2-TCMP ylide proceeded smoothly, to afford the corresponding *E*-2-thiazolyl-α,β-enones in high yield. Further reactions involving the Michael addition of sodium benzyloxide to the olefinic linkage, followed by cyclization of the adduct and conversion of the thiazolyl unit, led to the ulosonic acids.

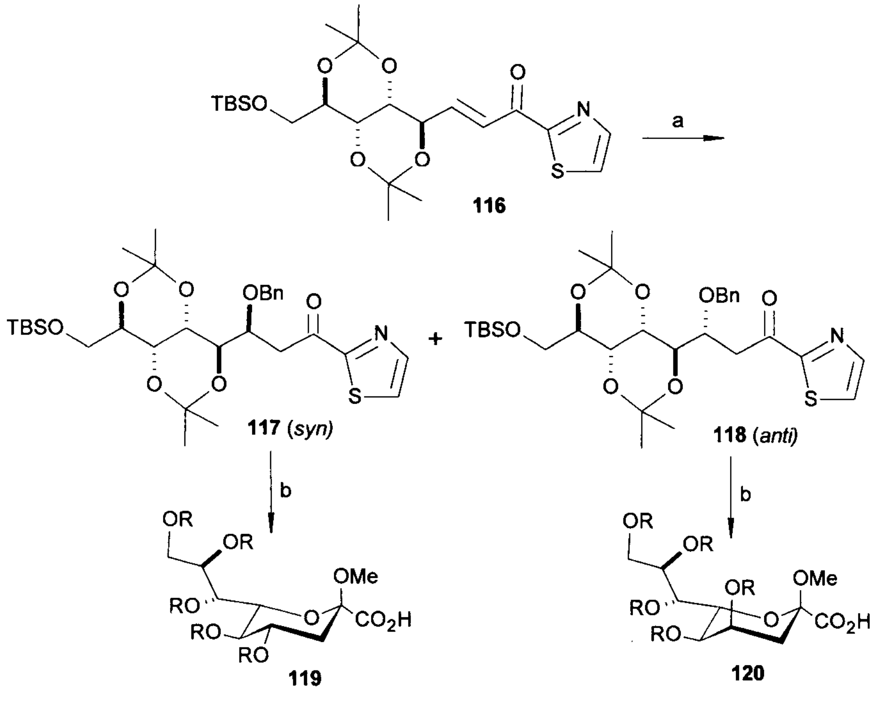



**Scheme (25).** Reagents: (a) $CHCl_3$, (b) BnONa, (c) 1. MeOH, HCl, 2. BnBr, NaH.

Approaching to the synthesis of DAH [96], a suitable protected tetrose-derived **110** was coupled with **94** to afford the olefin **111**. Conjugate

addition of sodium benzyloxide to the double bond gave the *syn* adduct **112** highly preponderating (*syn*/*anti* = 81:19). Removal of the acetonide residue was accompanied by cyclization, furnished **114** as a precursor of DAH (Scheme 25).

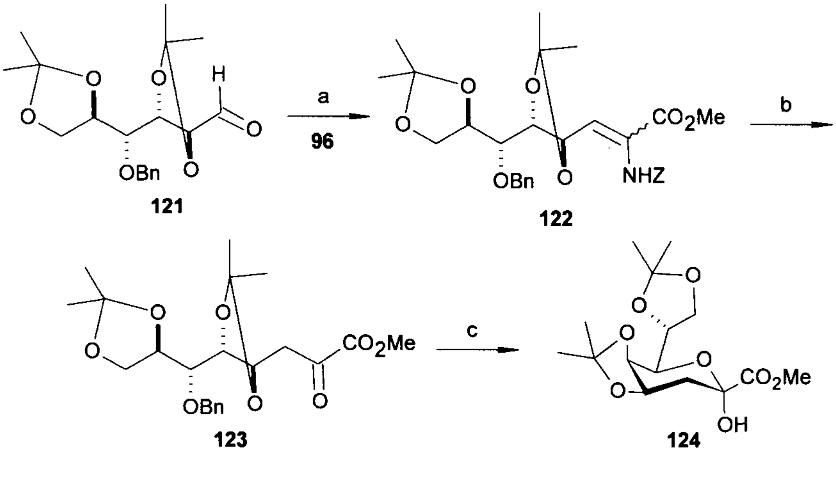
Following the above procedure the synthesis of KDN and 4-epi-KDN was accomplished [96]. α,β-Enone **116** derived from D-mannose upon reaction with sodium benzyloxide afforded *syn* **117** and *anti* **118** alkoxides in a (7:3) ratio (Scheme 26). Conversion of both the alkoxides to the final products was performed according to Scheme 26.




**Scheme (26).** Reagents: (a) BnONa, (b) 1. MeOH, HCl, 2. BnBr, NaH, 3. TfOMe, then $NaBH_4$, 4. $CuCl_2$, CuO, $H_2O$, 5. $Ag_2O$.

At about the same time Sato *et al.* synthesized KDO and KDN, utilizing Horner-Wittig reagent **96** and aldehydo D-mannose and D-*glycero*-D-*galacto*-heptose, respectively [97]. The synthesis proceeded through the α-dehydro amino acid derivatives. In the case of KDO it was produced from the ylide **96** and aldehyde **121** to give **122** as a mixture of

*E/Z* = (2:1) isomers, easy transformed to the corresponding α-oxoester **123**. The last one underwent an intramolecular cyclization under debenzylation conditions. In the same manner KDO was prepared in high yield.



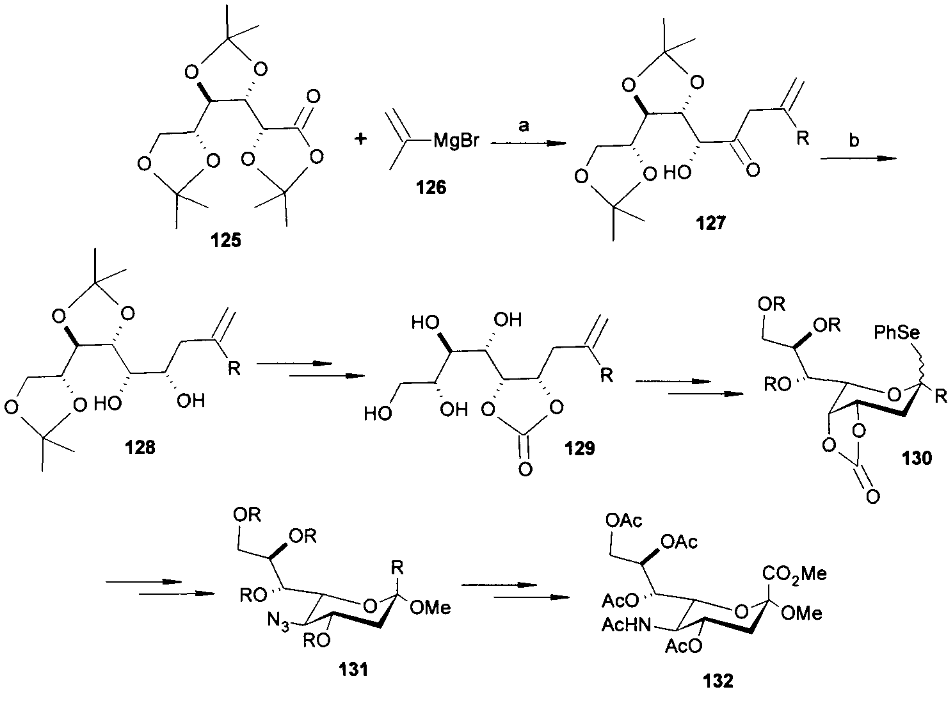

**Scheme (27).** Reagents: (a) NaH, (b) $H_2$, Pd/C, (c) $H_2$, $Pd(OH)_2$.

Horner-Emmons strategy found also the use for the synthesis of KDO in which methyl [2-*O*-(*tert*-butyldimethylsilyl)-α-dimethoxy phosphinyl] glyoxylate served as a substrate [98], however, the yield of the synthesis was rather modest.

## Syntheses *via* Grignard Reaction

An interesting, novel strategy towards the synthesis of Neu5Ac has been presented recently by Schmidt and Khadair [99]. It consists in the coupling of a D-gluconolactone derivative **125** with an allyl Grignard reagent **126**, to produce the corresponding nine carbon atoms molecule **127** (Scheme 28). Reduction of the carbonyl group proceeded highly stereoselectively in favor of the desired *erythro* isomer **128** (30:1). Protection of the vicinal hydroxyls as a cyclic carbonate allowed to remove the isopropylidene residue and subsequently the closure the hexopyranose ring. Introduction of the acetamido function at the proper C-5 position was succeeded *via* regioselective formation of the triflate,

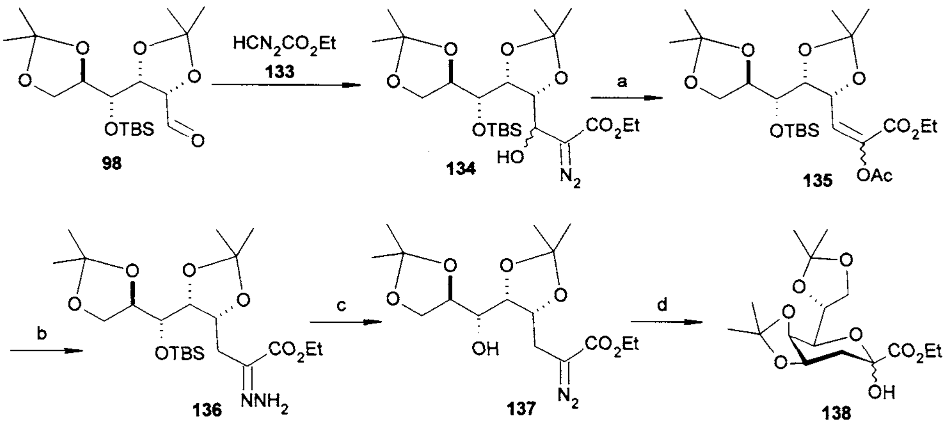
followed by its substitution with azido group to afford compound **131**, which was converted in five steps into the ester **132**.




**Scheme (28).** Reagents: (a) $Et_2O$, THF, (b) $Zn(BH_4)_2$.

In the course of a search for a new strategy of the chain elongation of the aldose aldehydes Lopez-Herrera *et al.* [100] have developed a method involving a reaction with the ethyl 2-diazoacetate. An example of this strategy, leading to KDO is illustrated in Scheme 29. As a substrate served the aldehyde **98** which on treatment with ethyl diazoacetate **133** provided the (3:2) diastereomeric mixture of the corresponding β-hydroxy-α-diazoesters **134**, separable by chromatography. Its acetylation gave a very labile enol acetate **135** which was stabilized *via* reaction with hydrazine to afford **136**. Further steps, involving oxidative cleavage of the hydrazone group, than cyclization using MCPBA furnished KDO derivative **138**.

Using this route DAH was also prepared [101] as well as its 2-deoxy counterpart.



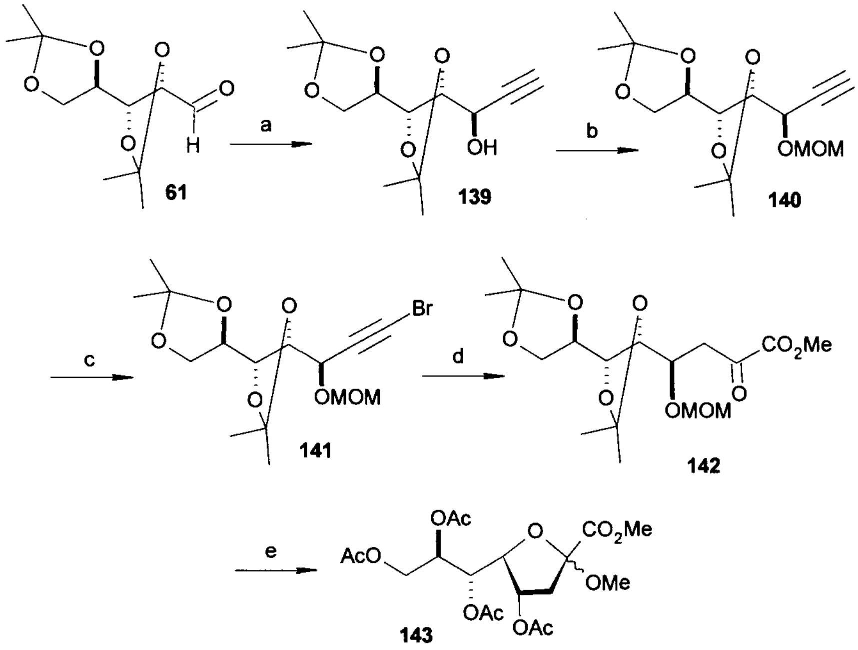

**Scheme (29).** Reagents: (a) 1. $Ac_2O$, py, 2. $Rh_2(OAc)_4$, (b) $(NH_2)_2$, MeOH, (c) 1. TBAF, 2. $MnO_2$, (d) MCPBA.

It is worth to note that β-acetoxy-α-diazoesters were also readily accessible through the reaction of monosaccharides in their hemiacetal form. However, such a reaction required the use of an equimolecular amount of diethylzinc creating likely very reactive zinc diazo-intermediates which react smoothly with aldehydes [102]. Applying this procedure KO and its 3-epimer were prepared, starting from 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose [101].

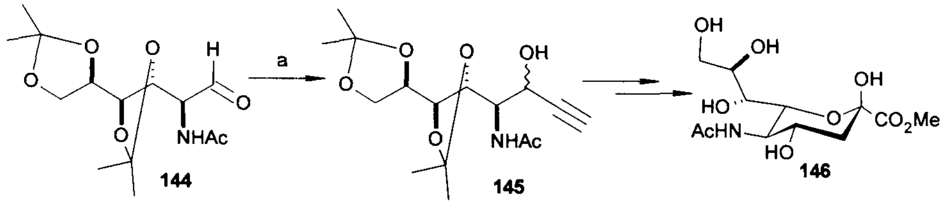
An original, and versatile route toward ulosonic acids has been recently elaborated by Wu *et al.* [103,104,105]. It based on a simple introduction of α-keto acid moiety *via* propargylation of suitable monosaccharides derived aldehydes and subsequent oxidation of the terminal alkynes. As it is exemplified by preparation of KDO, coupling of **61** with 3-bromopropyne gave the *anti*-adduct **139** (Scheme 30). Its bromination using NBS/$AgNO_3$ provided the bromoalkyne **140**, which on reaction with $KMnO_4$ afforded the desired α-keto acid ester, easily convertible into the anomeric mixture of KDO derivatives **143**. The yield of all intermediates were very high.

The same reaction sequence led to the Neu5Ac methyl ester **146** when an appropriate amino aldehyde **144**, prepared in eight steps from the gluconolactone, served as a substrate [104] (Scheme 31). Processing as above the alkyne **145** was obtained as a mixture of *syn*/*anti* adducts in a (74:11) ratio, the former being isolated by chromatography was then transformed into **146**.

By this course of reactions a series of 3-deoxy-2-ulosonic acids were synthesized, including DAH [105], DRH [105] and 4-epi-KDN [103].



**Scheme (30).** Reagents: (a) $C_3H_3Br$, Zn, DMF, $Et_2O$, (b) MOMCl, (c) NBS, $AgNO_3$, acetone, (d) $KMnO_4$, $NaHCO_3$, $MgSO_4$, (e) 1. HCl, MeOH, 2. $Ac_2O$, py.

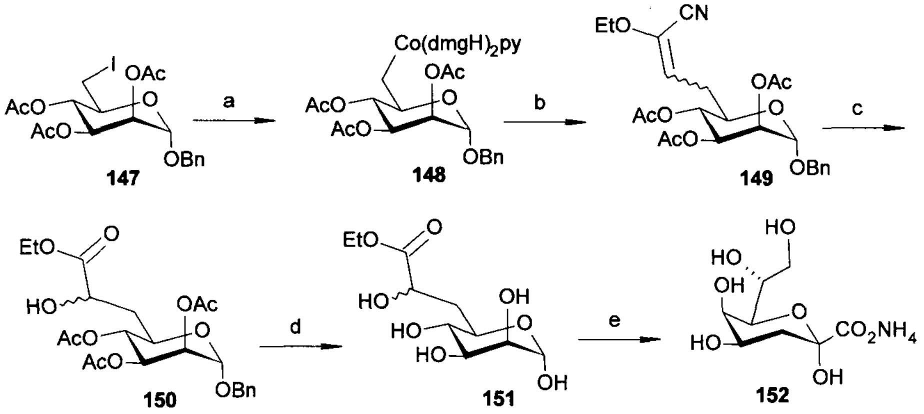


**Scheme (31).** Reagents: (a) $C_3H_3Br$, Zn, DMF, $Et_2O$.

## Radical Approaches

In contrast to the most synthetic strategies involving ionic elongation reactions at C-1 of aldoses, a synthesis of KDO based on the photochemically induced radical cross-coupling of α-ethoxyacrylnitrile with an alkyl cobaltoxime derivative at C-6 of D-mannose has been reported by Branchaud *et al.* [106]. Thus, treatment of 6-iodo-D-

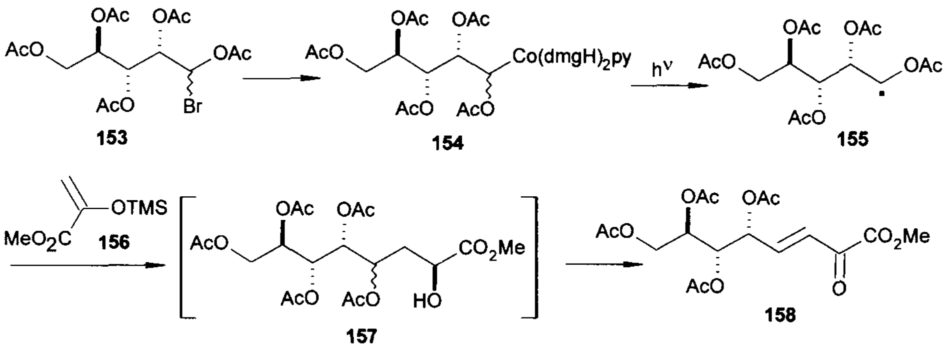
mannoside **147** with NaCo(dmgH)2py nucleophile afforded the corresponding 6-deoxy-6-cobalt(III)-dimethylglyoximatopyridine **148** which under photolysis with α-ethoxyacrylonitrile furnished the olefin **149**. Further steps of the synthesis are shown in Scheme 32.




**Scheme (32).** Reagents: (a) NaCo(dmgH)2py, MeOH, $H_2O$, (b) $CH_2$=(CN)OEt, hν, (c) $KMnO_4$, py, acetone, (d) 1. $H_2$, Pd/C, AcOH, AcOEt, 2. NaOH, MeOH, (e) 1. $NaBH_4$, Amberlite, 2. $V_2O_5$, $H_3PO_4$, $NaClO_3$, $H_2O$, 3. $NH_3$aq.

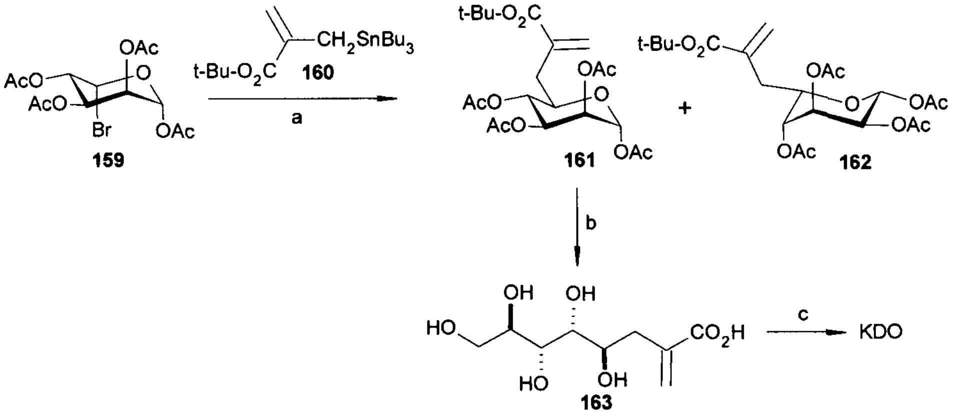
The above route seems to be rather wearing, giving the final product **152** in low yield.

A more convenient route to KDO *via* cobaltoxime reported by Giese *et al.* is represented by the reaction of the silylated pyruvate **156** with the arabinosyl cobaltoxime **154**, according to Scheme 33 [107]. This resulted in the formation of the precursor **158** ready for conversion to the KDO salt **152**.




**Scheme (33).**

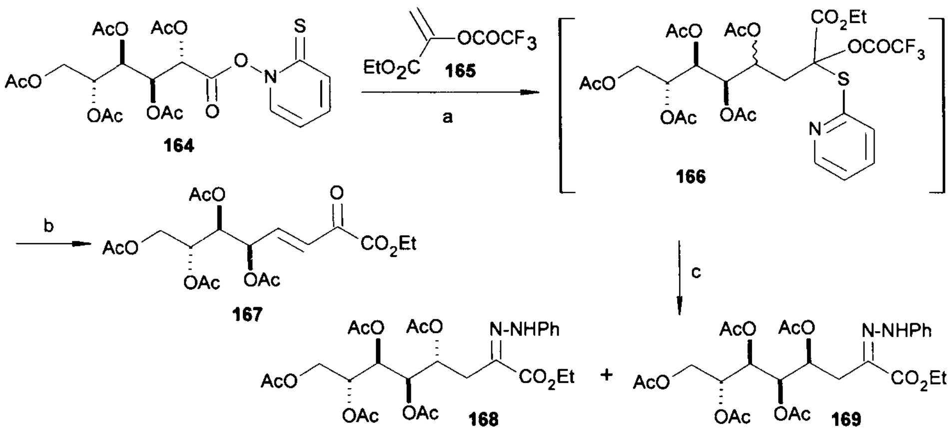
The radical elongation process was involved in the another synthesis of KDO which started from D-lyxose bromide **159** and alkene **160** [108], (Scheme 34). The reaction proceeded in dry benzene under reflux to give a (3:1) diastereoisomeric mixture of **161** and **162**. Conversion of the isomer **161** to the KDO salt required deprotection and reduction of the hemiacetal moiety, which led to **163**. Ozonolysis process subsequent by treatment with $NH_3$aq gave the desired KDO in high yield.




**Scheme (34).** Reagents: (a) AIBN, benzene, (b) 1. NaOHaq, 2. $NaBH_4$, (c) 1. $O_3$, THFaq, then $Me_2S$, 2. $NH_3$aq.

By an analogy to the radical syntheses above, the carbon chain elongation reaction was achieved by Barton *et al.* [109] in the reaction with ethyl α-trifluoroacetoxy acrylate **165**. Under radical conditions **165** was coupled with the ester **164** prepared from pentaacetyl gluconic acid and *N*-hydroxy-2-thiopiridone (Scheme 35) to afford an unstable intermediate **166**, readily convertible to the unsaturated α,β-keto acid **167**. The methoxymercuration-demercuration of **167** gave a low selectivity, providing the mixture of 3-deoxy-4-methoxy-D-*manno*- and D-*gluco*-octulosonic acids (4:3) in a moderate yield [110].

To improve this synthesis [109] the intermediate **166** was reacted with phenylhydrazine, to give the epimeric mixture of **168** and **169**. This idea based on an assumption that the hydrazone could be a better masking group of α-keto acid function [109]. Unfortunately, these derivatives, after conversion to the corresponding lactones, were unable to create the desired ulosonic acids.



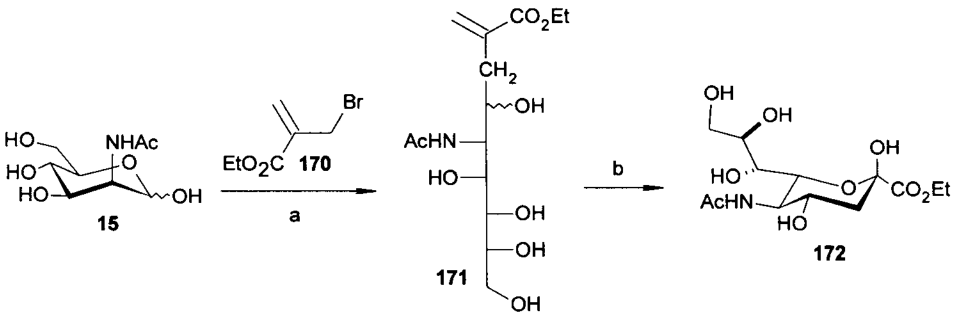

**Scheme (35).** Reagents: (a) DCC, hv, (b) $NaHCO_3$aq, (c) $PhNHNH_2$.

The synthesis *via* Barton esters succeeded when the thiopirydyl adduct was created from 2,3:4,5-di-*O*-isporopylidene ribonic acid as a substrate [109c]. Treatment of this adduct with aqueous $NaHCO_3$ gave an epimeric mixture of ethyl 4,5:6,7-di-*O*-isopropylidene-3-deoxy-D-*arabino*- and D-*ribo*-2-heptulosonates (3:1, respectively), the first one was further converted into DAH ethyl ester by use of Dowex (H+) resin in aqueous EtOH.

## Syntheses from Pyranoses

It is obvious that monosaccharides in their hemiacetal form are the best substrates if they are able for the introduction of α-keto acid unit at the anomeric center. These substrates do not require tedious multistep selective protection-deprotection procedures to liberate the carbonyl function. A series of reactions were examined, what succeeded in developing of diverse routes to the construction of 3-deoxy-ulosonic acids.

One of the best methods developed by Whitesides *et al.* was an indium mediated direct nucleophilic addition of ethyl 2-(bromomethyl)acrylate to unprotected aldoses in aqueous media [111] (Scheme 36). An other advantage of this reaction is that it can be compatible with a variety of functional groups [111,112]. The resulting allyl-compounds were produced in good yield. Ozonolysis, then cyclization of the resulting ketones afforded the corresponding ulosonic acids.



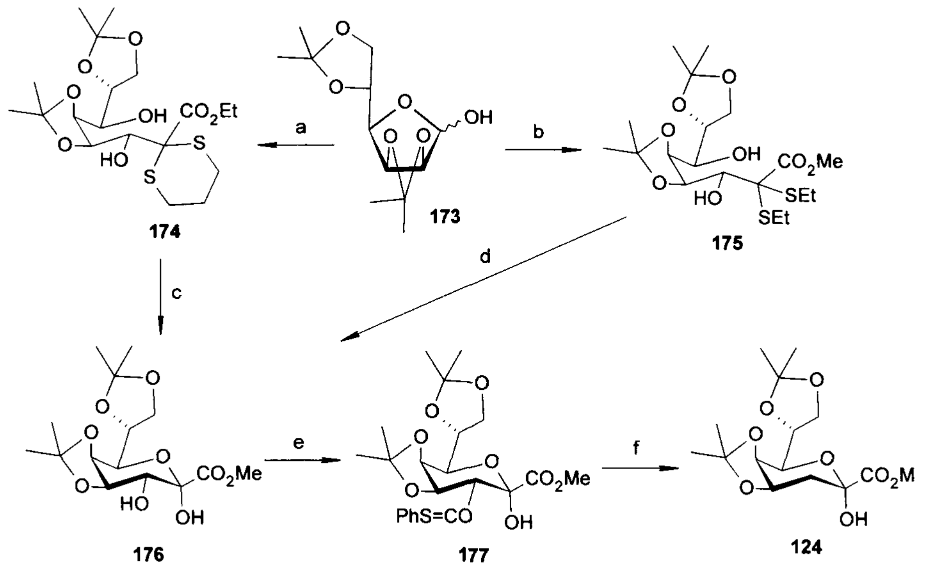

**Scheme (36).** Reagents: (a) In, EtOH, (b) $O_3$, MeOH, then $Me_2S$.

The stereochemical outcome of allylation reaction is very satisfying due to predominant creation of *threo* isomer. This selectivity has been interpreted on the basis of the Cram type chelation [113,114]. It consists in formation of the five membered chelate between the allyl, indium and α-OH group of aldose, followed by the attack of the allylic nucleophile at the aldehyde from the less hindered side, thus favoring the *syn* product [111-114]. Similarly, the other sugars *i.e.* D-ribose, D-xylose and D-arabinose have been reacted to achieve the appropriate ulosonic acids [112].

An influence of pH of the reaction medium on steric course was observed by Warwel and Fessner [114]. Thus, allylation of L-arabinose with ethyl 2-(bromomethyl)acrylate at pH 1-2, afforded KDO with full stereoselectivity [114]. The same reaction conditions used for the allylation of D-mannose furnished KDN as the only isomer [114].

Similarly, condensation of D-mannosamine, as well as D-mannose with methyl dimethyl[(3-bromopropen-2-yl)phosphate] [115], instead of ethyl (bromomethyl)acrylate, led to the corresponding Neu5Ac and phosphonate analogs with much higher stereoselectivity (*syn/anti* ~ 20:1).

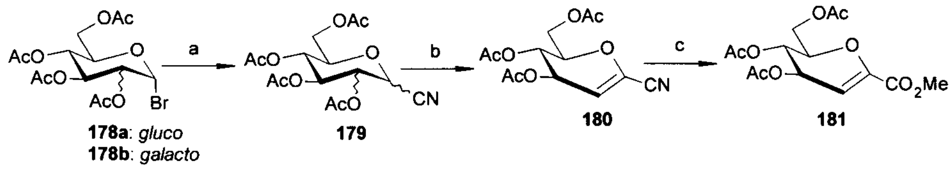
An alternative approach to the stereocontrolled synthesis of ulosonic acids was based on nucleophilic acylation of 2,3:5,6-di-*O*-isopropylidene-D-mannose **173** with a glyoxylate carboanion [116] derived from the mercaptal glyoxylate (Scheme 37). As a result the corresponding thio-octulosonates **174** and **175** were formed in 76% yield. Both compounds were converted to **176** using NIS (or NBS) and then MeONa. Applying Barton procedure [117] **176** was deoxygenated at C-3 to afford KDO derivative **124**.



Scheme (37). Reagents: (a) ethyl 1,3-dithiane-2-carboxylate, LDA, $MgBr_2$, (b) $(EtS)_2CHCO_2Me$, LDA, $MgBr_2$, (c) 1. NBS, acetone, 2. MeONa, MeOH, (d) NIS, acetone, (e) $PhC(Cl)=NMe_2Cl$, py, $H_2S$, AcOH, (f) $Bu_3SnH$, AIBN.

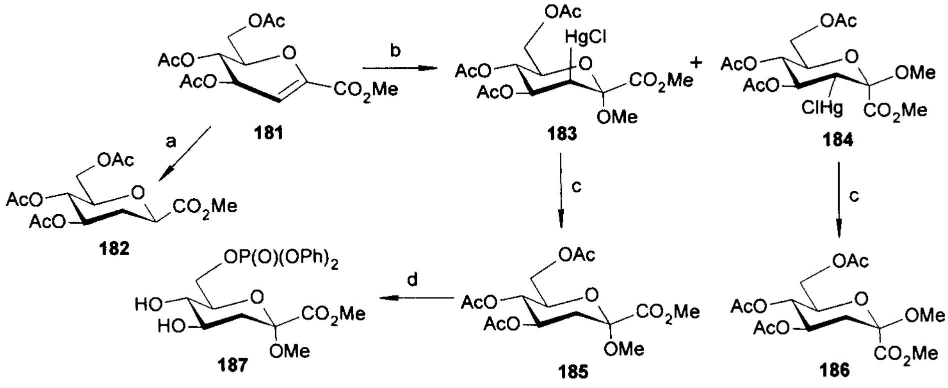
## Syntheses from Glycals

By application of various methods a series of ulosonic acids were readily accessed by homologation of glycal-type substrates [118]. One of the routes is illustrated by the preparation of 1-carbomethoxy glycals, suitable for functionalization to the isomeric heptulosonic acids (Scheme 38). Thus reaction of per-*O*-acetyl pyranosyl bromides **178** with $Hg(CN)_2$ gave the cyanoglycosides **179**, which in two steps were converted to the esters **181** [119,120].



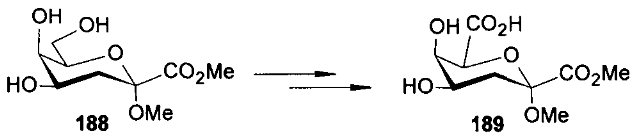
Scheme (38). Reagents: (a) $Hg(CN)_2$, (b) DBU, (c) 1. NaOH, EtOH, 2. $Ac_2O$, py, 3. $CH_2N_2$.

Catalytic hydrogenation of the olefinic linkage of the *arabino* isomer **181** led to 2-deoxy-DAH (**182**), whereas fully regioselective methoxymercuration-demercuration reaction, followed by phosphorylation of 7-OH group afforded DAHP derivative **187** (Scheme 39) [120b].



**Scheme (39).** Reagents: (a) $H_2$, Pd/C, (b) 1. $Hg(CO_2CF_3)_2$, MeOH, 2. KCl, (c) $Ph_3SnH$, AIBN, AcONa, (d) 1. $NaHCO_3$, MeOH, 2. $(PhO)_2POCl$, py.

The *lyxo* isomer of **181** submitted to methoxymercuration-demercuration reaction provided the epimeric compound **188** with full regio- and stereoselectivity [120a]. Its transformation to the corresponding heptulosaric acid derivative **189** (Scheme 40) was easily accomplished applying KBr/TEMPO/NaOCl assisted oxidation.



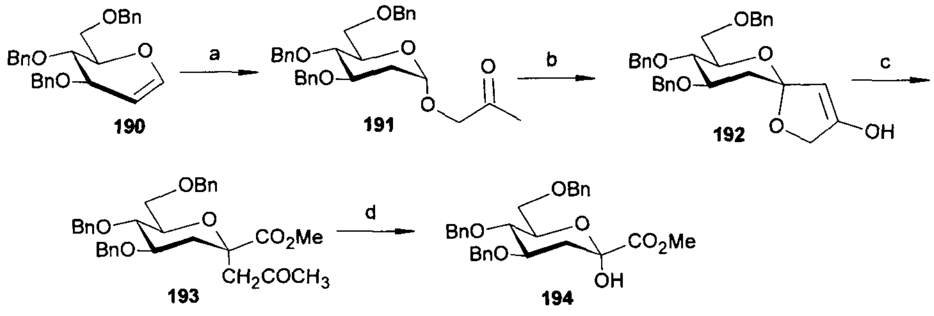
**Scheme (40).**

Based on the above route involving also an inversion of configuration at the desired (selected) carbon atoms, all the isomeric (*arabino*, *lyxo*, *xylo*, *ribo*) heptulosonic and heptulosaric acids have been achieved [120c].

It is worth to mention that the preparation of the intermediate **181** was also possible upon a direct introduction of the carbomethoxy group at C-1

of the protected glycals in a reaction with methyl chloroformate anion, generated with *tert*-BuLi [119,120c].

The studies on homologation of glycals towards synthesis of the ulosonic acids have been extended recently by the use of an unique sequence of glycosylation. Thus, alkylidene[1,5]-C-H insertion forming the spiroketal glycosides led to DAH ammonium salt in few steps shown in Scheme 41 [121].

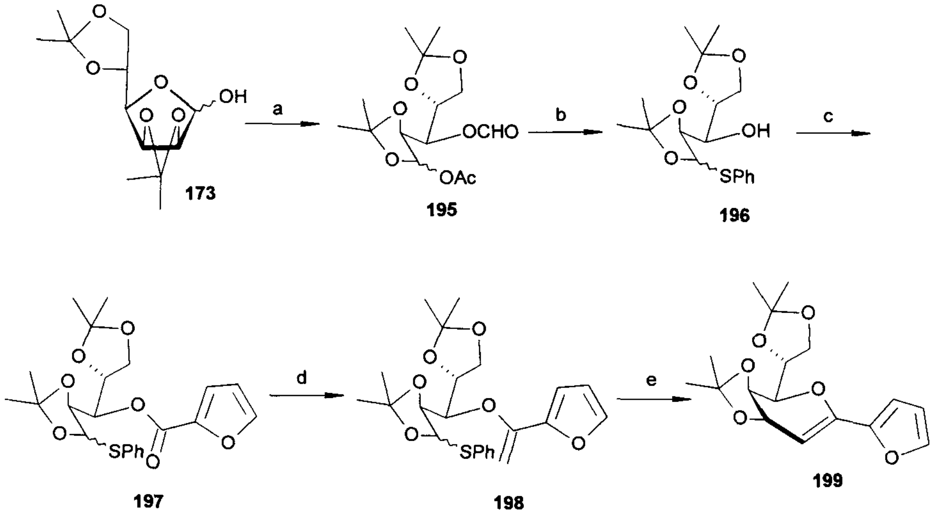



**Scheme (41).** Reagents: (a) 1. methallyl alcohol, TPP·HBr, 2. $OsO_4$, $NaIO_4$, $H_2O$, (b) $TMSCLiN_2$, then $NaHCO_3$, $H_2O$, (c) 1. $O_3/O_2$, then Zn, AcOH, 2. $NaClO_2$, $NaHPO_4{\cdot}H_2O$, *t*-BuOH, 2-methyl-2-butene, 3. $CH_2N_2$, (d) $SmI_2$, MeOH.

Thus, the glycosidation of **190** followed by oxidation led to the corresponding 2-oxopropyl glycosides **191**, which on treatment with [(trimethylsilyl)diazomethane]lithium at low temperature underwent insertion reaction to provide spiroketal derivative **192**. Subsequent ozonolysis of **192** liberated an unstable ketoaldehyde, which was immediately oxidized to the carboxylic acid, isolated as its methyl ester **193**. Removal of the 2-oxopropyl group was accomplished using $SmI_2$, to afford DAH derivative **194**.

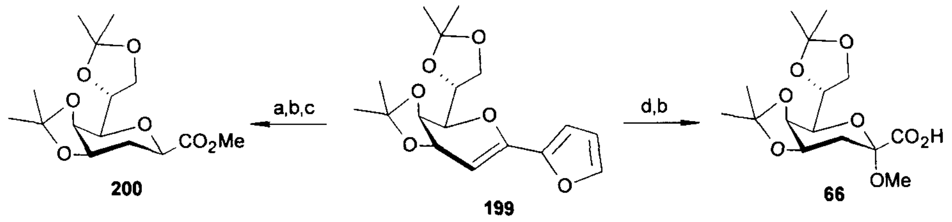
By essential the same route KDO was prepared starting from 2,3:5,6-di-*O*-isopropylidene-α-D-*manno*-furanose [121].

Quite recently, a new access to KDO from 1-thio-1,2-*O*-isopropylidene acetal **196** has been reported [122]. Its condensation with 2-furoic acid, followed by the Tebbe methylenation afforded **198** (Scheme 42). Cyclization process was mediated by methyl triflate. Thus obtained 1-furano glycal derivative **199** was converted into KDO derivative **66** and its 2-deoxy-α-analog **200** in a reaction sequence shown in Scheme 43 [122].



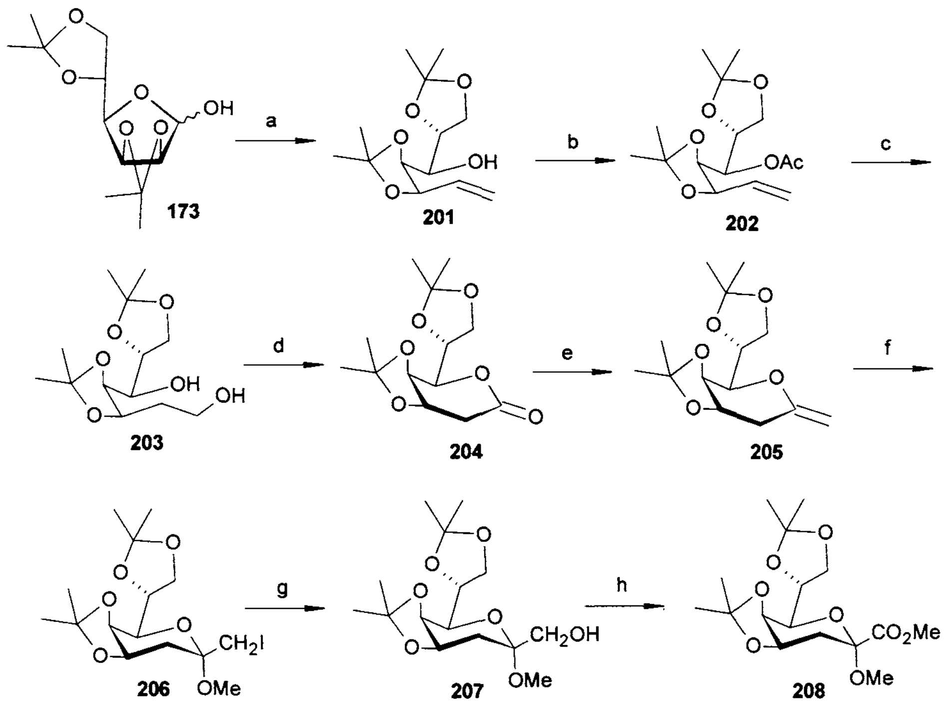

**Scheme (42).** Reagents: (a) iodosobenzene diacetate, $I_2$, (b) 1. PhSH, $BF_3{\cdot}Et_2O$, 2. NaOMe, MeOH, (c) 2-furoic acid, DCC, DMAP, (d) Tebbe reagent, (e) MeOTf, DTMBMP, MS.

Methanolysis of **199**, followed by oxidative degradation of the furan ring proceeded smoothly, providing KDO derivative **66**. Transformation of **199** into 2-deoxy-KDO required the hydrogenation in the first step leading after several transformation to the methyl ester of the resulted acid.



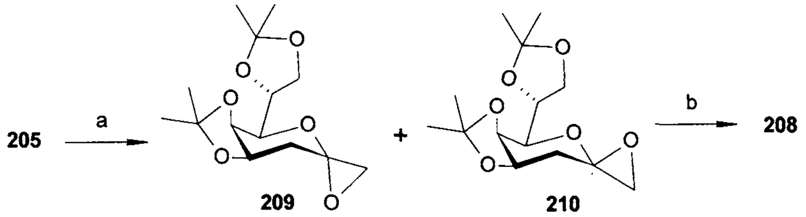

**Scheme (43).** Reagents: (a) $H_2$, Pd/C, (b) $RuO_2$, $NaIO_4$, (c) $CH_2N_2$, (d) MeOH, CSA.

An interesting strategy towards synthesis of KDO proceeding through *exo*-glycals as a key intermediate has been reported by Sinaÿ [123] (Scheme 44).



**Scheme (44).** Reagents: (a) $Ph_3P{=}CH_2$, THF, HMPA, (b) $Ac_2O$, py, (c) 9-BBN, then $H_2O_2$, NaOH, (d) PCC, (e) Tebbe reagent, (f) *t*-BuOK, $I_2$, MeOH, (g) 1. CsOAc, HMPA, 2. MeONa, MeOH, (h) 1. $(COCl)_2$, DMSO, 2. TEA, $NaOCl_2$, $H_2O_2$, 3. $CH_2N_2$.

The synthesis began with the Wittig methylenation of 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose to give **201**. Hydroboration-oxidation reaction led to the lactone **204** which with the Tebbe reagent afforded the desired exoglycal **205**. Making use of the double bond the α-glycosylation was performed, and iodomethyl group generated in **206** was transformed into the $CO_2Me$ as it is shown in Scheme 44, thus leading to KDO derivative **208** in good yield.



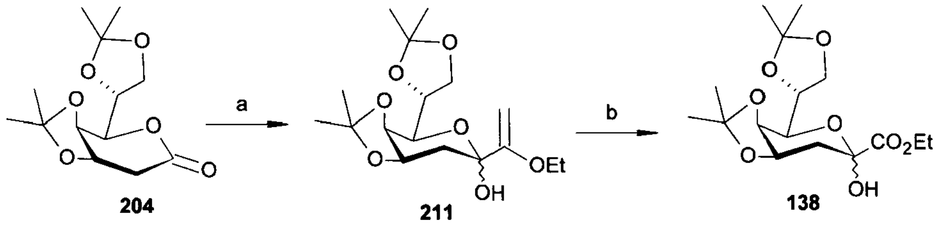
**Scheme (45).** Reagents: (a) DMDO, (b) MeOH, $ZnCl_2$.

The *exo*-glycal **205**, when converted to the labile epoxide **209/210**, led to the glycoside **208** in one step by $ZnCl_2$ promoted reaction with methanol [124].

## Syntheses Based on the Addition Reactions to the Carbonyl Group of Aldonolactones

It is known, that 2-deoxy lactones are able to react directly with a suitable acyl equivalent, to produce the corresponding precursors of ulosonic acids. To this end a range of addition reactions to lactones by use of "masked" carboxylate group equivalent were explored [125]. Among them 2-furyllithium [125a], 2-thiazoyllithium [125b], tris(methylthio)methyllithium and 2-(1,3-dithianyl)-lithium [125c] were exploited to give the addition products in satisfactory yield. However, liberation of the carboxyl function in the most cases was tedious, or even failed [125-127]. In contrast, reaction of the lactone **204** with 1-ethoxyvinyllithium, followed by ozonolysis succeeded, furnishing the anomeric mixture of KDO derivatives **138** in high yield (Scheme 46) [128].

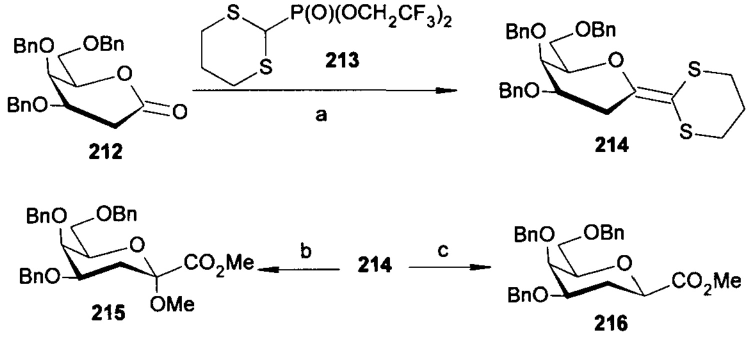
Looking carefully at the current literature we have learned that the ketene dithioacetal chemistry, widely used for one-carbon homologation of carbonyl compounds, has never been utilized for the construction of ulosonic acids [129]. Besides, ketene dithioacetals, readily available from ketones and aldehydes *via* the reaction with the ylides generated from substituted dithioacetals in the Peterson, Wittig or Horner-Emmons olefination, have never been synthesized from lactones.




**Scheme (46).** Reagents: (a) ethoxyvinyllithium, (b) $O_3$, then $Me_2S$.

The Horner-Emmons olefination procedure turned out to be the method of choice for the preparation of the desired ketene dithioacetals

[130]. The key features of this approach is summarized in Scheme 47 (for the *lyxo* isomer).




**Scheme (47).** Reagents: (a) KHMDS, (b) NBS, MeOH, (c) 1. $LiBH_4$-TMSCl, 2. NBS, THF, $H_2O$, 3. $CH_2N_2$.

In all cases but one ketene dithioacetals **214** were formed as the sole products. The yield of the reaction (82-62%) dependent on the configuration of the starting lactone. Due to the application of 2-[bis(2,2,2-trifluoroethoxy)phosphoryl]-1,3-dithiane (**213**), possessing a strong electron withdrawing group stabilizing likely dithianyl anion [131], the competitive β-elimination reaction in sugar ring [132] could be effectively avoided. Oxidative hydrolysis of ketene dithioacetals with NBS-MeOH in $CH_2Cl_2$ resulted in a clean and stereoselective formation of α-methyl 3-ulosonates (**215**) in ~ 80% yield.

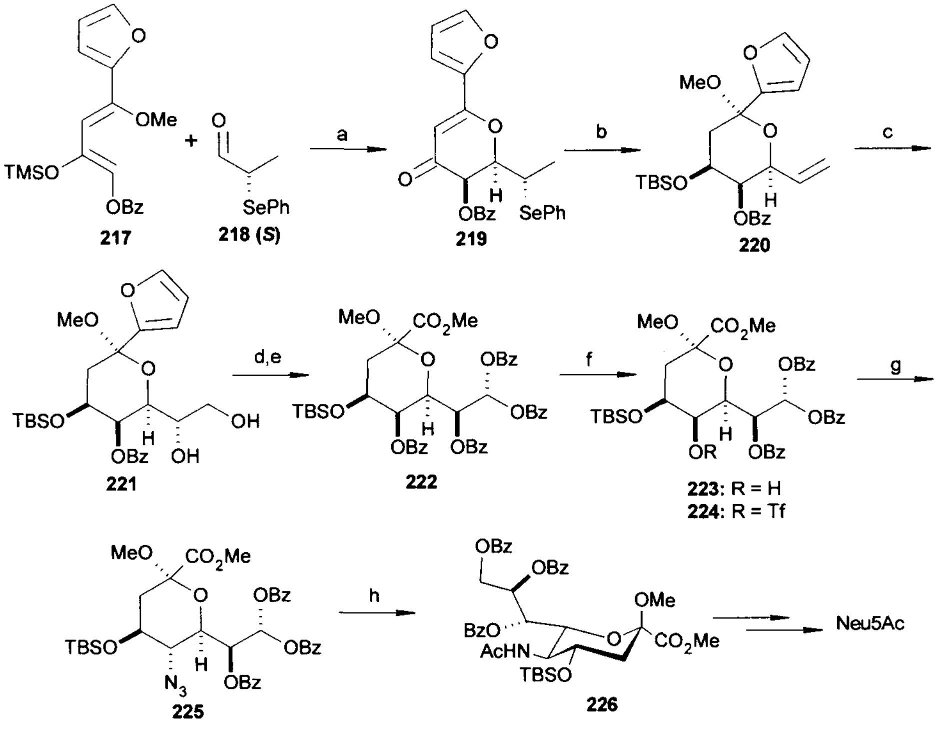
The remarkable feature of this approach is that it could be utilized towards direct, stereospecific α-glycosylation. This was illustrated by addition of sugar alcohols to ketene dithioacetals, promoted by TMSOTf, to reach after oxidative hydrolysis of the dithianyl residue, the corresponding α-disaccharides in high yield [130a]. The steric course of the glycosylation reaction, is possibly controlled by the influence of the dithianyl group for the attack of a nucleophile on one side.

This ketene dithioacetal protocol was employed to deliver all the isomeric 3-deoxy-heptulosonic and heptulosaric acids, as well as their 2-deoxy counterparts [130]. Further, by this route the synthesis of KDO and 2-deoxy-KDO was accomplished [133]. This methodology was also utilized for direct construction of naturally occurring KDO disaccharides [134].

## TOTAL SYNTHESES

All previously described approaches to 3-deoxy-2-ulosonic acids started from advanced carbohydrate precursors, and thus they are not easily applicable to the synthesis of structurally diverse analogues. This concerns chemical syntheses relying on sugar-derived stereocenters and enzymatic ones, providing only very limited access to some naturally occurring compounds. In contrast, *de novo* syntheses starting from simple, achiral compounds offer much more flexible alternative approaches to synthesis of unnatural ulosonic acid analogues.

The first total synthesis of Neu5Ac from non-carbohydrate precursor has been reported by Danishefsky *et al.* in 1988, who used a hetero Diels-Alder reaction between a diene and an aldehyde to establish the pyranoid ring [135] (Scheme 48).

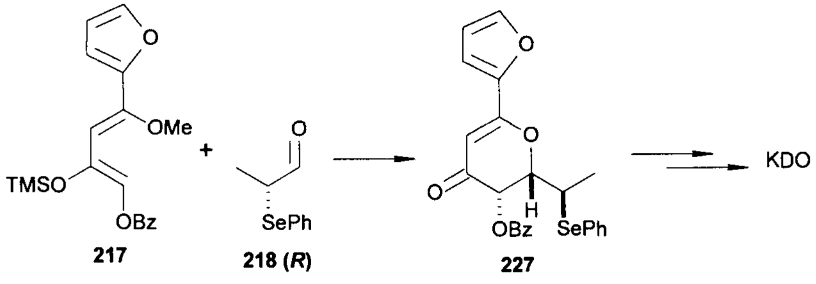



**Scheme (48).** Reagents: (a) 1. $BF_3{\cdot}OEt_2$, 2. TFA, (b) 1. $NaBH_4$, $CeCl_3$, 2. CSA, MeOH, 3. TBSOTf, 2,6-lutidine, 4. $H_2O_2$, (c) $OsO_4$, NMO, (d) 1. $Pb(OAc)_4$, 2. $(CF_3CH_2O)P(O)CH_2CO_2Me$, KHMDS, 18-c-6, 3. $OsO_4$, NMO, 4. $LiEt_3BH$, 5. BzCl, (e) 1. $NaIO_4$, $RuO_2$, 2. $CH_2N_2$, (f) 1. HF, 2. $Tf_2O$, py, (g) $Bu_4NN_3$, (h) Lindlar catalyst.

A critical element of that synthesis was the concept of using a furan ring as a surrogate for a carboxylic acid function. The furyl diene **217** reacted with *S* enantiomer of 2-(phenylseleno)propionaldehyde with high regiospecificity providing *cis* and *trans* isomers in a (5:1) ratio (Scheme 48). Desired *cis* product was transformed into **220** with 81% yield in four steps. Stereospecific hydroxylation led to a Neu5Ac precursor **221**, which in turn was oxidized with ruthenium tetraoxide causing demasking of the carbonyl function. The resultant intermediate **222** was then transformed into **226** over several steps, involving deprotection, substitution, inversion of configuration at C-5 and reduction of $N_3$ residue.

An useful consequence of the furan surrogate was its ability to promote exchange reactions of an anomeric methoxyl group with a variety of primary "sugar alcohols".

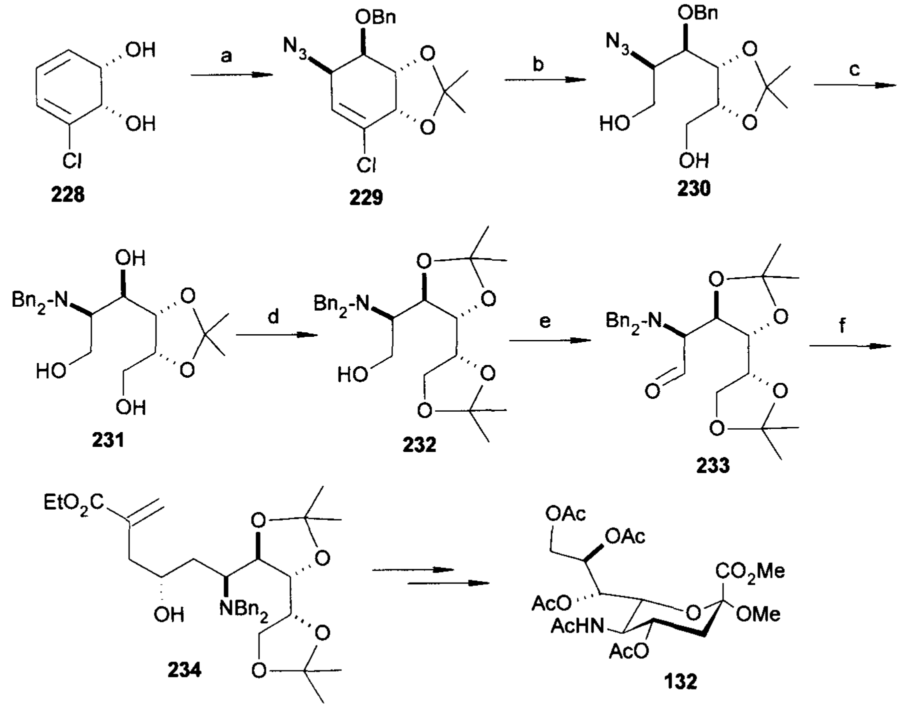
It is worth to note that enantiomer of **221** is an intermediate in the KDO synthesis. For the purpose of synthesizing KDO *R* enantiomer of aldehyde **218** was used as a starting material (Scheme 49). Thus obtained dihydropyranes **227** were converted into KDO in nine steps elaborated previously during Neu5Ac synthesis.




**Scheme (49).** Danishefsky's KDO synthesis

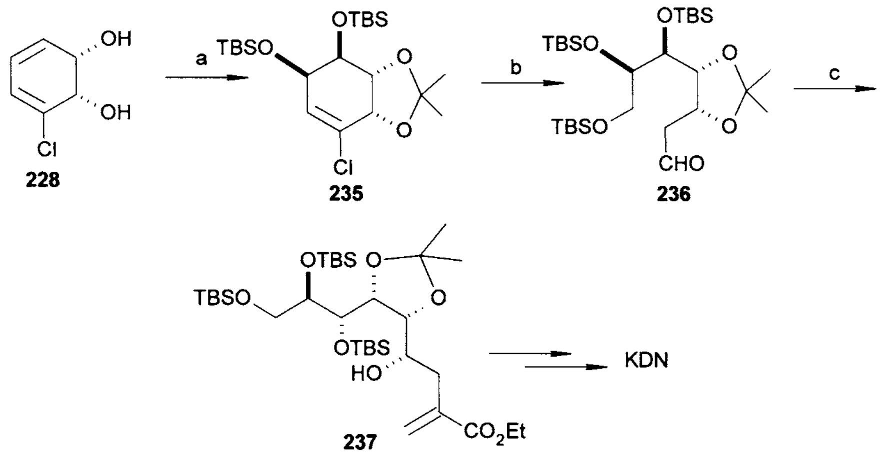
In Banwell's *de novo* synthesis of Neu5Ac [136] *cis*-1,2-dihydrocatechol **228**, a product of microbial oxidation of chlorobenzene, has been converted into a protected form of Neu5Ac *via* a fifteen steps reaction sequence (Scheme 50). Synthesis started from azido alcohol **229**, obtained from catechol **228** by an established procedure [137]. This was subjected to ozonolytic cleavage and a reductive work-up to afford diol **230**. Protection-deprotection reaction sequence led to alcohol **232** which was then oxidized to D-mannosamine derivative **233** using the Swern protocol. Condensation with the organozinc reagent derived from

bromoacrylate gave the *anti*-addition product **234** in 85% yield. A sequential five steps transformation led to the desired Neu5Ac in low yield.




**Scheme (50).** Reagents: (a) Ref. 137, (b) $O_3$, MeOH, then $NaBH_4$, (c) 1. $H_2$, Pd/C, 2. BnBr, $K_2CO_3$, (d) $Me_2CO$, TfOH, (e) Swern oxidation, (f) bromoethyl acrylate, Zn.

Similar protocol was applied in the first total synthesis of KDN from non-carbohydrate sources [138]. Catechol derivative **228** was transformed into protected tetraol **235**, according to the literature method [139] (Scheme 51). This, in turn, underwent smooth ozonolytic cleavage to give after reductive work-up the ester alcohol, which was converted, over three steps, into the unstable D-mannose derivative **236**. Reaction of compound **236** with pyruvate anion equivalent gave a *syn* product **237**, accompanied by the another isomer (3:2). Conversion into KDN was performed in two steps involving ozonolytic cleavage of the double bond, then deprotection with concomitant cyclization using TFA-water mixture.



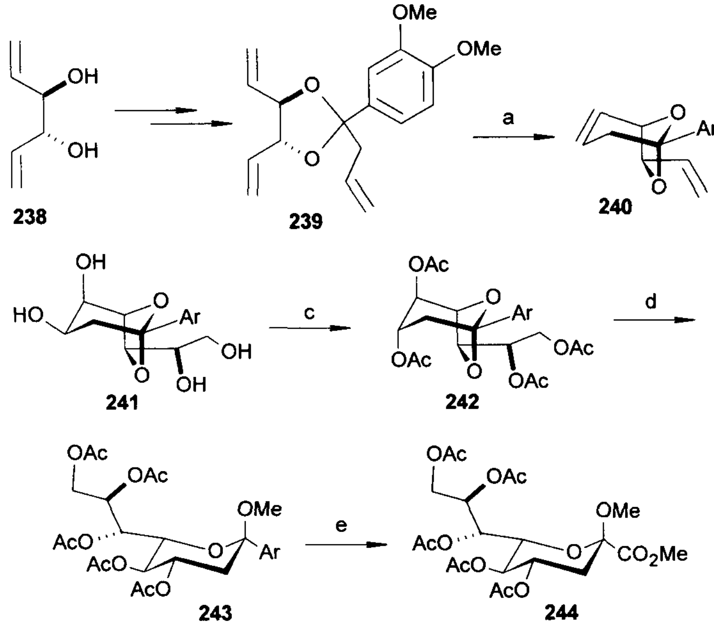

**Scheme (51).** Reagents: (a) Ref. 139, (b) 1. $O_3$, MeOH, then $NaBH_4$, 2. TBSOTf, 2,6-lutidine, 3. LAH, 4. Swern oxidation, (c) bromoethyl acrylate, Zn.

An original routes to KDN [140] and Neu5Ac [141] was designed by Burke *et al.* In these syntheses a rigid 6,8-dioxabicyclo[3.2.1]octane template was served for the stereoselective introduction of both oxygen and nitrogen functionalities, giving race to the expected compounds (Scheme 52). Approaching to the preparation of KDN, in the first step the diene diol **238** was converted into the triene **239** in high yield. Treatment of **239** with Grubbs ruthenium catalyst resulted in a ring-closing metathesis reaction. Subsequent double Sharpless asymmetric dihydroxylation (SAD) provided the tetraol **241**. Tin-assisted tosylation reaction, followed by nucleophilic substitution inverted configuration at C-4 revealing the natural configuration of KDN. Methanolysis of precursor **242** and final unmasking of the carboxylic function delivered the desired KDN in 45% overall yield (Scheme 52).

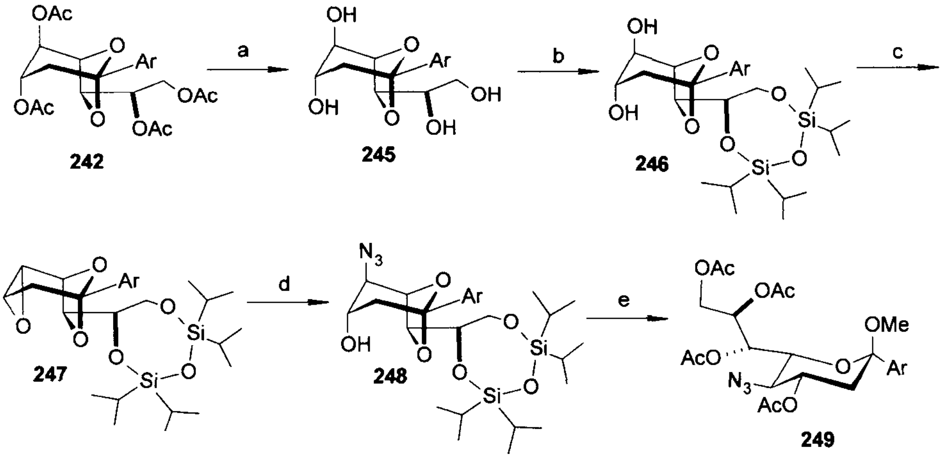
Conformationally defined bicyclic acetal template and readily differentiated hydroxyl in the 2,7-anhydro sugar analogue **241** makes this synthesis flexible enough for preparation various KDN derivatives.

Utilization of **242** for preparation of Neu5Ac [141] required an introduction of the acetamido function. With this aim **242** was suitably blocked to give **246**. Selective 5-OH-*exo* tosylation and subsequent treatment with NaOMe in MeOH allowed to form α-epoxide **247**. Opening of the oxirane ring by use of $NaN_3$ afforded the azidoalcohol **248** as a sole product, easily transformed into methyl glycoside **249** as it is

shown in Scheme 53. Demasking of carboxyl function and reductive acetylation of azide led to protected form of Neu5Ac [141].



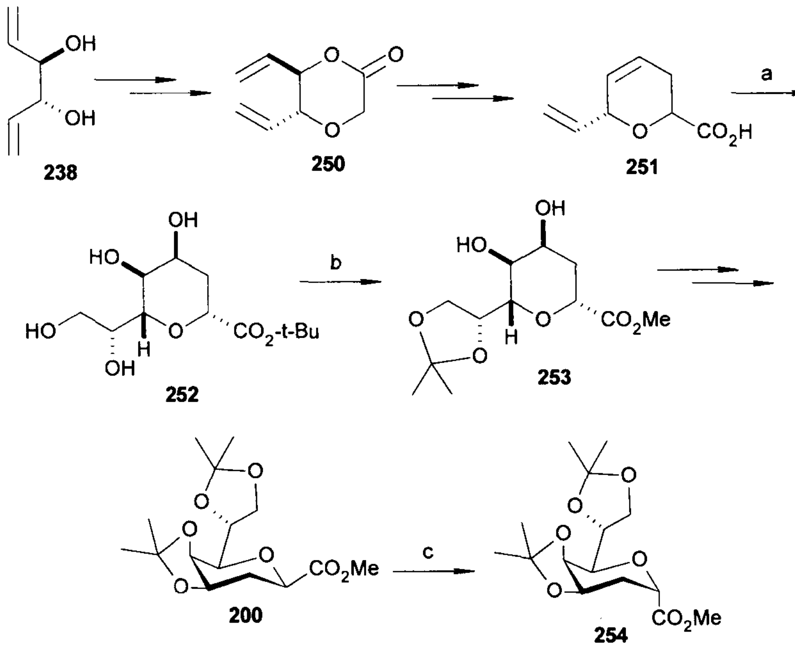

Scheme (52). Reagents: (a) Grubbs catalyst, (b) $OsO_4$, NMO, $(DHQ)_2AQN$, (c) 1. TsCl, $Bu_2SnO$ cat., 2. $Ac_2O$, TEA, 3. CsOAc, 18-c-6, (d) 1. MeOH, $H^+$, 2. $Ac_2O$, TEA, (e) 1. $RuCl_3$ cat, $NaIO_4$, 2. $TMSCH_2N_2$, MeOH.




Scheme (53). Reagents: (a) NaOMe, MeOH, (b) $TIPSCl_2$, base, (c) 1. TsCl, TEA, 2. NaOMe, MeOH, (d) $NaN_3$, (e) 1. Amberlite ($H^+$), MeOH, 2. TBAF, 3. $Ac_2O$, TEA.

Among various modifications of KDO, 2-deoxy-β-KDO which is believed to be inhibitor of CMD-KDO synthetase has attracted great interest as a potential antibiotic [142]. Another importance of 2,3-dideoxy ulosonic acids comes from the possibility of using them as the precursors of glycosyl donors.

The developed by Burke route to ulosonic acid has been also utilized for the synthesis of 2-deoxy compounds [143] (Scheme 54).



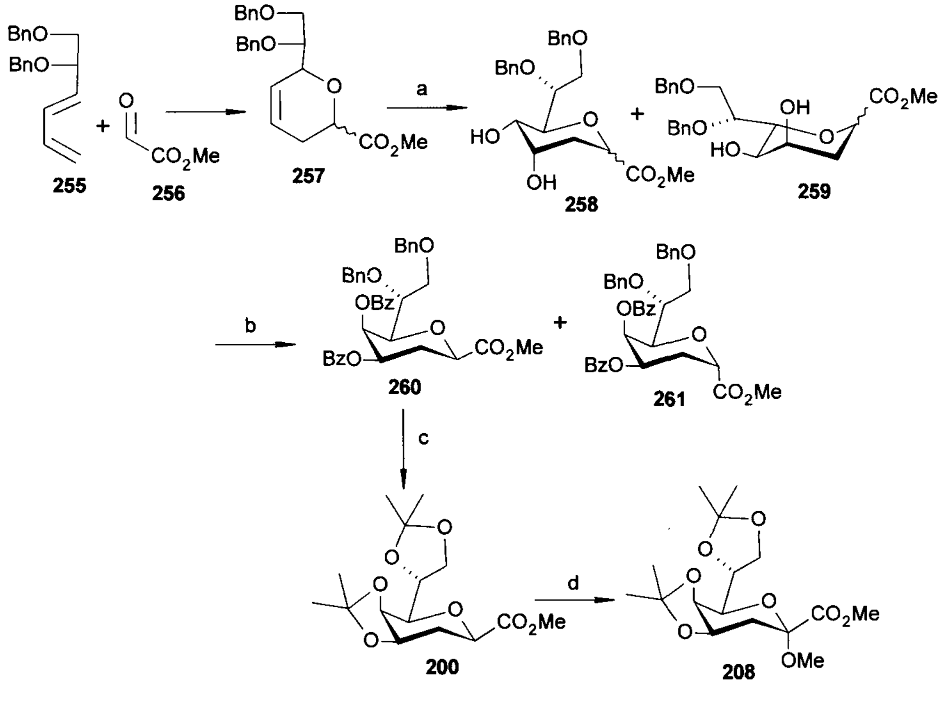
Scheme (54). Reagents: (a) 1. *t*-butyl 2,2,2-trichloroacetimidate, 2. $OsO_4$, NMO, $(DHQ)_2AQN$, (b) 1. CSA, MeOH, 2. DMP, CSA, (c) LDA (60% + 18% **39b**).

Starting from diene diol **238** a dessymetrization was performed by its conversion to the corresponding 1,4-dioxanone. Irreland-Claisen rearrangement of **250** provided the 6-vinyldihydropiran-2-carboxylate template **251** which was oxidized by double Sharples asymmetric dihydroxylation. Under all examined conditions dihydroxylation occurred from the undesired β-face leading to tetraol **252**. Transesterification of **252** was followed by differentiation of the two vicinal diols by selective formation of the C-7, C-8 acetonide residue. Unprotected hydroxyls were inverted leading, after protective groups manipulation, to **200**, an intermediate in Augé synthesis of KDO [144]. Epimerization of **200** was

succeeded *via* lithium enolate, which oxidation was proved to be useful for the conversion of **254** to KDO.

The hetero Diels-Alder reaction has been used to access the eight [144,147], seven [145] and nine [146,148] carbon 2,3-dideoxy-ulosonic acid skeleton in which the C-2-C-3 and C-6-O-6 bonds were created in cycloaddition step.

To take advantage of the synthetic intermediate on the way to KDO, Augé developed a new strategy, based on an aqueous hetero Diels-Alder reaction followed by a dihydroxylation of newly created double bond [144] (Scheme 55).

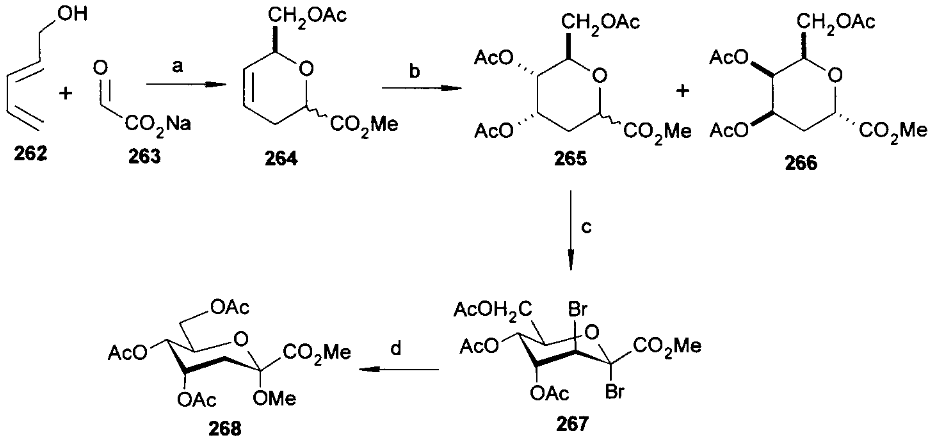



**Scheme (55).** Reagents: (a) $OsO_4$, NMO, (b) 1. $Tf_2O$, py, 2. $Bu_4NOBz$, 3. isolation of α-anomer, (c) 1. MeONa, MeOH, 2. $H_2$, Pd/C, 3. DMP, acetone, pTSA, (d) 1. LDA, 2. PhSSPh, 3. TBS, $THF_{aq}$.

In the course of the synthesis, the mixture of benzylated cycloadduct **257**, obtained from diene **255** and glyoxylic acid ester **256**, was hydroxylated with osmium tetraoxide to afford mixture of unseparable diols **258** (hydroxyalation onto *si* face of the *si* cycloadducts) and **259** (hydroxyalation onto *re* face of the *re* cycloadducts). This mixture

required a bis-inversion to attain the configuration of KDO. This process and subsequent protective group manipulation gave rise to 2-deoxy-α-KDO, which was sulfonylated *via* the enolate to yield β-thioglycoside, the hydrolysis of which led to KDO (Scheme 55).

Analogous cycloaddition reaction of sodium glyoxylate, in water, onto penta-2,4-dienol has been used for synthesis of seven carbon 2-deoxy-ulosonic acid analogues as depicted in Scheme 56 [145].



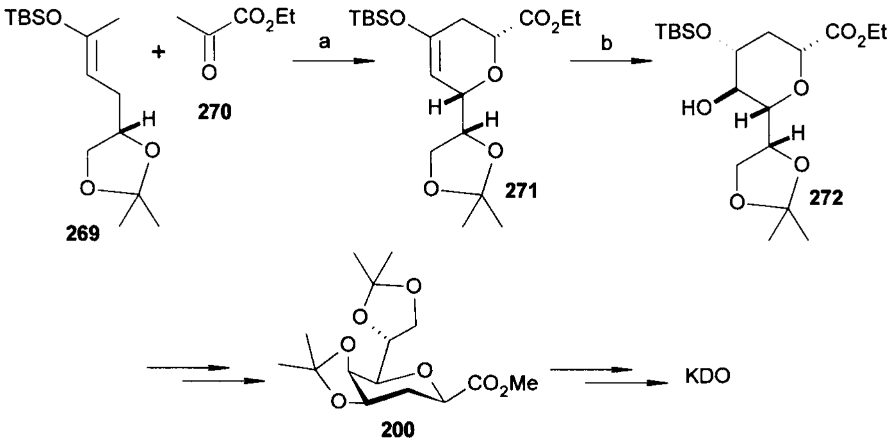

Scheme (56). Reagents: (a) 1. MeI, 2. $Ac_2O$, py, (b) $OsO_4$, NMO, (c) NBS, (d) 1. MeOH, $Hg(CN)_2$, $HgCl_2$, 2. $Bu_3SnH$, AIBN.

The major products **265** and **266** arise from an attack of oxidant *anti* to the pyranosic oxygen atom and substituent in position 6. Activation of the anomeric carbon was achieved through NBS mediated C-2-C-3 dibromination. Dibromides **267** have been shown to serve as glycosyl donors in Koenigs-Knorr type reaction, since blocking the 3-axial position prevent the competitive elimination, often observed during glycosylation reactions of 2-ulosonic ester halides [149]. The racemic 4-*epi*-DAH acetylated esters **268** were thus obtained.

The stereochemical outcome of the hetero Diels-Alder reaction of the erythrose based diene with sodium glyoxylate was further rationalized by preparing the same compounds *via* decarboxylation of 2-carboethoxy-2-deoxy-2-ulosonic acid esters, obtained by cycloaddition with diethyl ketomalonate [146]. A range of 2-nonulosonic acid derivatives - KDN analogues - were prepared including protected form of 2-deoxy-KDN,

2,3-dibromo-KDN and its C-4-epimer as well as D-*glycero*-D-*manno*- and D-*glycero*-D-*allo*-2-nonulosonates [146]. Nevertheless, both previously presented original protocols suffer from the low selectivity and poor yield of the cycloaddition step.

Interesting exploration of this methodology presented Wu [147], where hetero Diels-Alder reaction between an electron-rich diene **269** and ethyl glyoxylate was assisted with salen-Co(II) catalyst (Scheme 57).

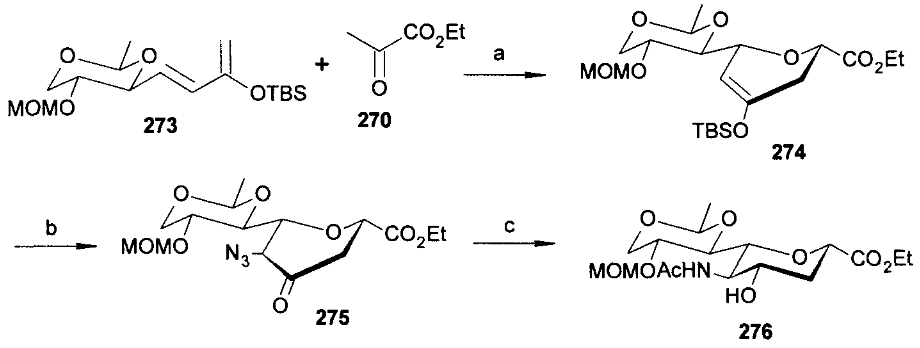



**Scheme (57).** Reagents: (a) 1. salen-Co(II) cat., (b) 1. $BH_3$, THF, 2. $H_2O_2$, $OH^-$.

Desired compound **271**, having the same stereochemistry at C-6 as KDO, was obtained with good selectivities (*endo*/*exo* = 93/7, *si*/*re* = 85/15). Further elaboration of **271** was done as shown in Scheme 57, starting with introduction of a hydroxyl group at C-5. An inversion of configuration (oxidation and reduction according to the Luche method) and protective group exchange led to KDO precursor **200**.

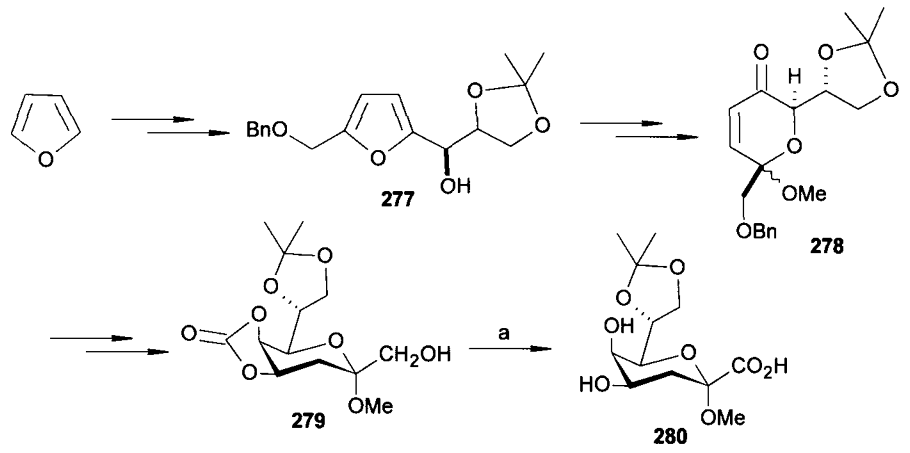
An efficient approach to Neu5Ac, 2-deoxy-β-Neu5Ac and 4-*epi*-2-deoxy-β-Neu5Ac based on salen-Co(II) complex catalyzed hetero Diels-Alder reaction was presented more recently [148]. The key features of this approach are summarized in Scheme 58. Condensation of diene **273**, prepared in five steps from D-glucose, and the ethyl glyoxylate furnished the cycloaddition product **274**. The next key step of the synthesis was to introduce an amino group at C-5, what was achieved on oxidative azidation reaction way. Conversion of **275** to the corresponding acetamide using a modification of Rosen's method and reduction of the

ketone with $NaBH_4$ afforded the desired *anti* product **276**, which is protected 2-deoxy-β-Neu5Ac form. Oxidation of the lithium enolate of protected 2-deoxy-β-Neu5Ac using previously described procedure [143] gave 2-α-OH product (Neu5Ac).




**Scheme (58).** Reagents: (a) 1. salen-Co(II) cat., (b) $NaN_3$, CAN, (c) 1. $CH_3COSH$, 2. $NaBH_4$.

12-Steps KDO synthesis from furan presented Martin *et al.* [150]. The reaction started from addition of 2-lithiofuran to isopropylidene-D-glyceraldehyde (Scheme 59).

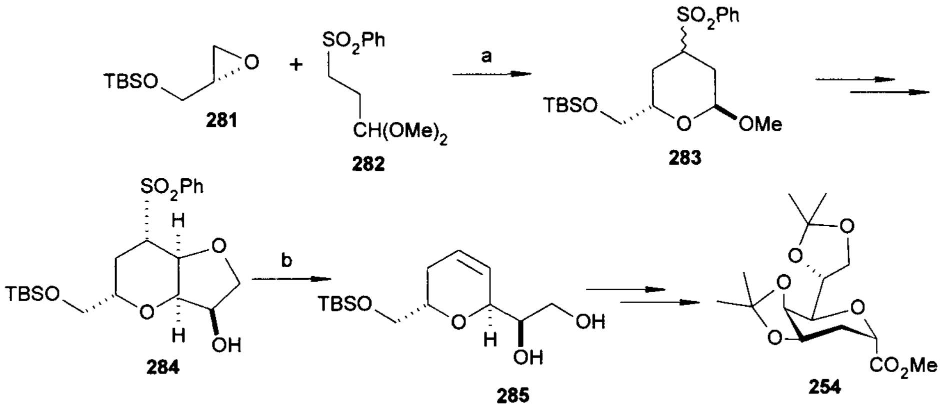



**Scheme (59).** Reagents: (a) 1. Swern oxidation, 2. $Ag_2O$, $OH^-$.

Metalation of the product followed by alkylation with benzyl chloromethyl ether and hydroxyl deprotection provided **277**. The key

transformation of the synthesis entailed sequential oxidative processing of **277** with *t*-BuOOH in the presence of catalytic amount of $VO(acac)_2$ and *O*-methylation of the intermediate hemiacetal moiety to furnish the α-methyl glycoside **278**. Stereoselective 1,2-reduction of **278** and functionalization of the double bond followed by the oxidation of the intermediate primary alcohol moiety at C-1 of **279** delivered partially protected KDO **280**.

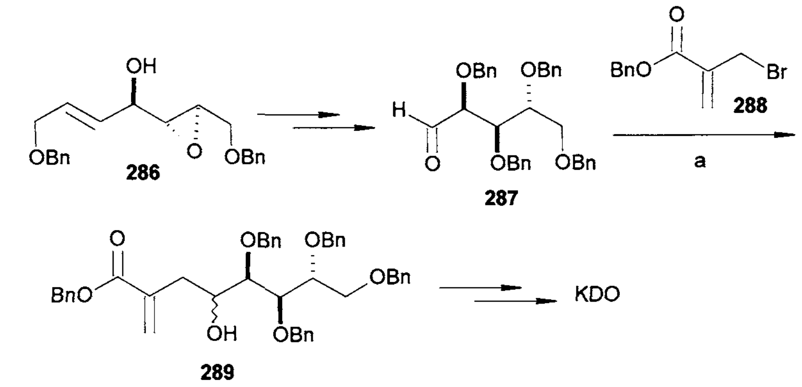
Different concept of 2-deoxy-KDO synthesis was presented by Craig [151]. The synthetic route started from epoxide **281** and 3,3-dimethoxy-1-(phenylsulfonyl)-propane which were transformed, after several manipulation, to bicyclic alcohol **284** (Scheme 60).




**Scheme (60).** Reagents: (a) 1. BuLi, 2. TFA, (b) Na(Hg).

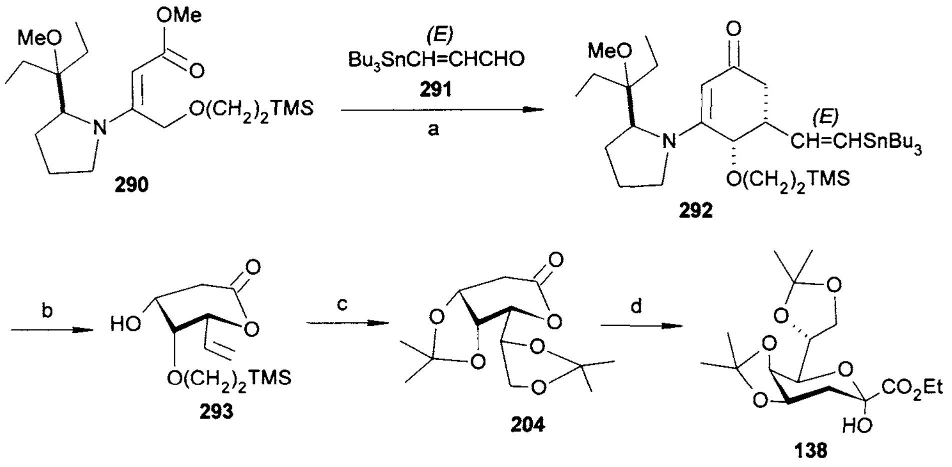
In order to cleave the bicyclic *C*-glycoside to the desired functionalized dihydropyran, **284** was exposed to sodium amalgam. *Syn*-dihydroxylation of **285** ($OsO_4$-NMO) gave a mixture of diastereomeric tetraols, which after separation, protection-deprotection procedure and oxidation of the product C-1 alcohol, using generated *in situ* ruthenium tetraoxide, gave the acid **254**.

Asymmetric Sharples epoxidation was applied by Schreiber to the synthesis of epoxide **286** being starting material for KDO synthesis [152] (Scheme 61). Aldehyde **287**, obtained from **286**, underwent aldol type condensation with α-(bromomethyl)acrylate to 8-carbons skeleton of KDO and its C-4 epimer (1:1) mixture.




**Scheme (61).** Reagents: (a) $CrCl_2$.

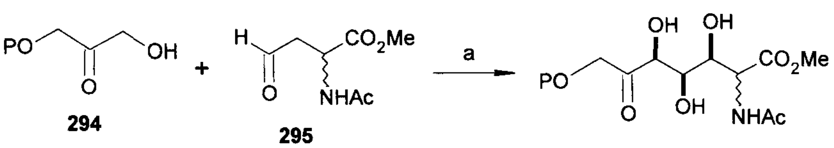
Asymmetric *syn*-selective aldol reaction of four carbons enolates (with attached chiral auxiliary) **290** and acrolein equivalent **291** was the key step in the Pettus synthesis of KDO [153]. Condensation adduct **292** was converted into the hydroxy lactone **293**, which in turn after silyl deprotection and diol side chain installation gave lactone **204**. Final addition of a carboxylic acid to the lactone carbonyl group of **204** was done with α-ethoxy vinyllithium. Reductive ozonolysis of the surrogate provided the ethyl ester **138** (Scheme 62).




**Scheme (62).** Reagents: (a) LDA, (b) 1. HCl, 2. $CeCl_2$, $NaBH_4$, 3. $Ag_2CO_3$, (c) $BF_3{\cdot}Et_2O$, PPTS, acetone, 3. $OsO_4$, NMO, 4. PPTS, acetone, (d) 1. EVL, 2. $O_3$, DMSO.

Although the synthesis of seven carbon 3-deoxy-ulosonic acids are not as often presented as "higher" ones, there are some noteworthy examples published.

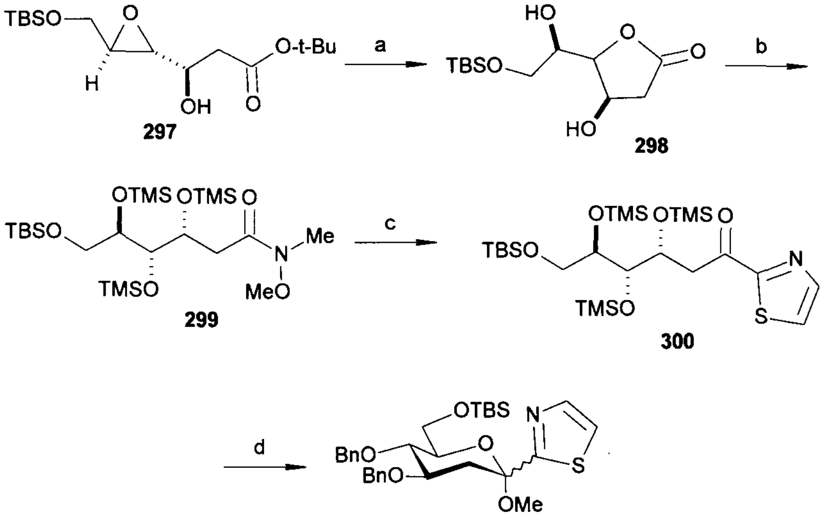
Enzyme assisted aldol condensation was the key step of the synthesis of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (Scheme 63) [154]. In the four steps synthesis of DAHP Whitesides started from *N*-acetyl-D/L-aspartate β-semialdehyde and dihydroxyacetone phosphate. The required *threo* stereochemistry was generated by using commercial rabbit muscle aldolase as catalyst.




294 295 296

**Scheme (63).** Reagents: (a) rabbit muscle aldolase.

In a Gorrichton's synthesis [155] a selectively protected 3-deoxy-D-arabino-2-heptulosonic acid was prepared in six steps from non-carbohydrate precursor with 19% overall yield. The used methodology is outlined in Scheme 64.




297 298 299 300 301

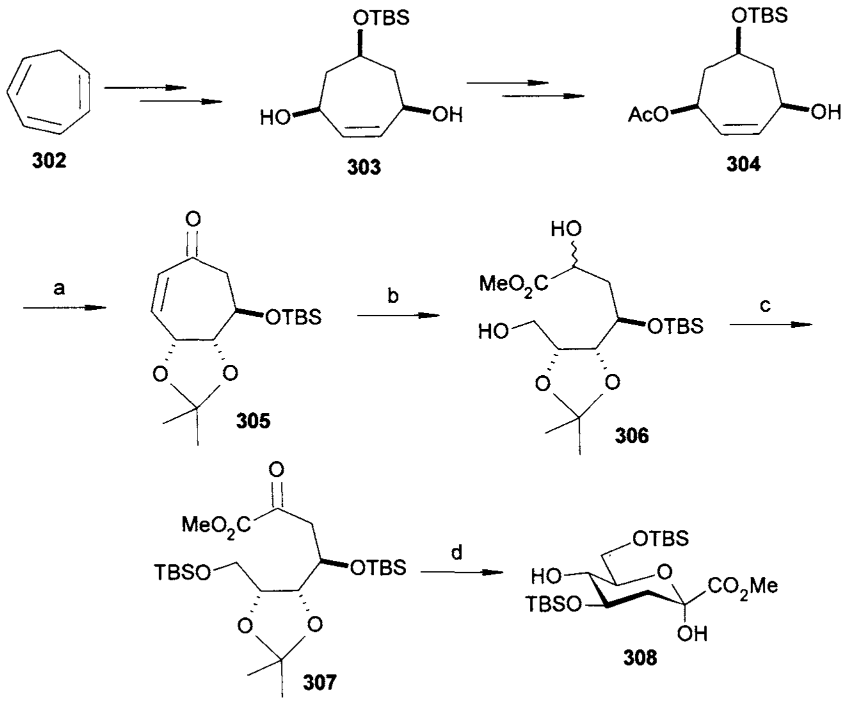
**Scheme (64).** Reagents: (a) Zn, TMS, (b) 1. MeNHOMe-HCl, $AlCl_3$, 2. TMSCl, HMDS, py, (c) thiazole, BuLi, (d) 1. PTSA, MeOH, 2. BnBr, NaH.

A Lewis acid mediated stereocontrolled lactonisation of α,β-epoxy-aldehyde **297** followed by a two-step procedure: synthesis of Weinrab's amide **299** and lithiothiazole nucleophilic attack allowed the introduction of the masked aldehyde frame in **300**. All attempts through the nucleophilic attack by a carbonyl (or formyl) equivalent on the lactone **298** failed to succeed. Cleavage of the trimethylsilyl protections and then benzylation of the hydroxyl functions gave compound **301** as a mixture of anomers. The major α-anomer was then subjected to one-pot thiazole to formyl transformation according to known procedure [82]. Oxidation of the resulting aldehyde with wet $Ag_2O$ and purification gave the heptulosonic acid derivative.

A more direct access to heptulosonic acid analogues have been described on [4+3] carbon atoms incorporation [156]. The Mukaiyama reaction of *trans*-α,β-epoxyaldehydes with ethyl 2-(trimethylsililoxy)-2-propenoate, in the presence of the Lewis acid – $BF_3$ etherate – led to the direct synthesis of a mixture of 5- and 6-fluoroheptulosonic analogues in their pyranosidic or furanosidic form [156].

A one step synthesis of heptulosonic and octulosonic bicyclic precursors by using *cis*-α,β-epoxyaldehydes have been also described [157].

A fully stereocontrolled total synthesis of protected DAH utilizing cycloheptatriene as a non-carbohydrate achiral starting material has been presented [158]. Cycloheptatriene was transformed into monoacetate **304** according to a previously reported reactions sequence which includes an enzymatic asymmetrization of the *meso*-diol **303** with *Pseudomonas cepacia* lipase, in isopropenyl acetate. Monoacetate **304** was transformed into the α,β-unstaturated ketone **305** which after several transformations (incl. bromination, Luche reduction and ozonolysis) gave ester **306** as a mixture of epimeric alcohols at C-2. Regioselective protection of the terminal hydroxyl group as the silyl ether and PDC oxidation of the secondary alcohol yielded protected heptulosonic acid **308** possessing the desired *arabino* stereochemistry. Acid catalyzed removal of the acetonide gave cyclized DAH derivative **308**.




**Scheme (65).** Reagents: (a) 1. TBSCl, Im, 2. $OsO_4$, NMO, 3. DMP, PTSA, 4. KOH, MeOH, 5. MsCl, TEA, 6. PTSA, MeOH, 7. $(COCl)_2$, DMSO, then TEA, (b) 1. $Br_2$, 2. $NaBH_4$, $CeCl_3$, 3. $O_3$, (c) 1. TBSCl, TEA, 2. PDC, (d) TsOH, MeOH.

## REFERENCES


[1] Lindberg, B. *Adv. Carbohydr. Chem. Biochem.*, **1990**, *48*, 279.

[2] (a) Lindberg, B; Samuellsson, K.; Nimnich, W. *Carbohydr. Res.*, **1973**, *30*, 63; (b) Kondo, S.; Zähringer, U.; Rietschel, E.T.; Hisatsune, K. *Carbohydr. Res.*, **1989**, *188*, 99.

[3] Vinogradov, E.V.; Knirel, Y.A.; Shashkov, A.S.; Gorin, S.E.; Vustina, T.F.; Soyfer, V.S.; Esopov, S.E.; Lisak, L.V.; Kochetkov, N.K. *Bioorg. Khim.*, **1988**, *14*, 1214.

[4] Kawahara, K.; Brade, H.; Rietschel, E.T.; Zähringer, U. *Eur. J. Biochem.*, **1987**, *163*, 489.

[5] Unger, F.M. *Adv. Carbohydr. Chem. Biochem.*, **1981**, *38*, 323.

[6] Haslam, E. Shikimic Acid Methabolism and Methabolites, Wiley, New York.

[7] (a) Lorkiewicz, Z.; Derylo, M.; Russa, R.; Skorupska, A.; Urbanik-Sypniewska, T. *Acta Microbiol. Polon.*, **1993**, *42*, 219; (b) Russa, R.; Urbanik-Sypniewska, T; Shashkov, A.S.; Banaszek, A.; Zamojski, A.; Mayer, H. *System. Appl.*

*Microbiol.*, **1996**, *19*, 1; (c) Weibgen, U.; Russa, R.; Yokota, A.; Mayer, H. *System. Appl. Microbiol.*, **1993**, *16*, 177.
[8] Brade, H.; Rietschel, E.T. *Eur. J. Biochem.*, **1985**, *153*, 249.
[9] Stevenson, T.T.; Darvill, A.G.; Albersheim, P. *Carbohydr. Res.*, **1989**, *179*, 269.
[10] Becker, E.; Hard, K.; Melkonian, M.; Kamerling, J.P.; Vliegenthart, J.F.G. *Eur. J. Biochem.*, **1989**, *82*, 153.
[11] (a) Knirel, Y.; Kochetkov, N.K. *FEMS Microbiol. Rev.*, **1987**, *46*, 381; (b) Knirel, Y.A.; Kochetkov, N.K. *Biochemistry,* **1994**, *59*, 1325; (c) Zähringer, U.; Knirel, Y.A.; Lindler, B.; Helbig, J.H.; Sonesson, A.; Marre, R.; Rietschel, E.T. in Bacterial Endotoxin Lipopolysaccharides from Genes to Therapy; Levin, J.; Alvin, C.P.; Munford, R.S.; Redl, H. Eds; Wiley-Liss; New York, **1995**, 113; (d) Helbig, J.H.; Lück, C.P.; Knirel, Y.A.; Zähringer, U. *Epidemiol. Infect.*, **1995**, *115*, 71.
[12] (a) Kiefel, M.J.; von Itzstein, M. *Chem. Rev.*, **2002**, *102*, 471; (b) Angata, T.; Varki, A. *Chem. Rev.*, **2002**, *102*, 439; (c) Schauer, B. *Adv. Carbohydr Chem. Biochem.*, **1982**, *40*, 131.
[13] Schauer, R.; Kamerling, J.P. in Glycoproteins II; Montrenil, J.; Vliegenthart, J.F.G.; Schachter, H. Eds; Elsevier, Amsterdam, **1997**, 243.
[14] Gil-Serrano, A.M.; Rodriguez-Carvajal, M.A.; Tejero-Mateo, P.; Espartero, J.L.; Menendez, M.; Corzo, J.; Ruiz-Sainz, J.E.; Benendia-Claveria, A.M. *Biochem. J.*, **1999**, *342*, 527.
[15] Castric, P.; Cassels, F.J.; Carlson, R.W. *J. Biol. Chem.*, **2001**, *276*, 26479.
[16] (a) Moll, H.; Knirel, Y.A.; Helbig, J.W.; Zähringer, U. *Carbohydr. Res.*, **1997**, *304*, 91; (b) Knirel, Y.A.; Moll, H.; Helbig, J.H.; Zähringer, U. *Carbohydr. Res.*, **1997**, *304*, 77.
[17] Varki, A. *Glycobiology,* **1993**, *3*, 97; (b) Collins, B.E.; Fralich, T.J.; Itonori, S.; Ichikawa, Y.; Schnaar, R.L. *Glycobiology,* **2000**, *10*, 11.
[18] Kitazume, S.; Kitajima, K.; Inoue, S.; Troy, F.A.II; Cho, J.-W.; Lennarz, W.J.; Inoue, Y. *J. Biol. Chem.*, **1994**, *269*, 22712.
[19] Nadano, D.; Iwasaki, M.; Endo, S.; Kitajima, K.; Inoue, S.; Inoue, Y. *J. Biol. Chem.*, **1986**, *261*, 11550.
[20] Inoue, S.; Lin, S.-L.; Chang, T.; Wu, S.-H.; Yao, C.-W.; Chu, T.-Y.; Troy, F.A.II; Inoue, Y. *J. Biol. Chem.*, **1998**, *273*, 27199.
[21] Wong, C.-H,; Whitesides, G.M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.
[22] Drauz, K.: Waldmann, H. *Enzyme Catalysis in Organic Synthesis*; VCH Publishers: New York, 1995.
[23] Fessner, W.-D., Walter, Ch. *Top. Curr. Chem.*, **1996**, *184*, 97.
[24] Toone, E.J.; Simon, E.S.; Bednarski, M.D.; Whitesides, G.M. *Tetrahedron,* **1989**, *45*, 5365.
[25] Drueckhammer, D.G.; Hennen, W.J.; Pederson, R.L.; Barbas, C.F., III; Gautheron, C.M.; Krach, T; Wong, C.-H. *Synthesis,* **1991**, 499.

[26] David, S.; Augé, C.; Gautheron, C. *Adv. Carbohydr Chem. Biochem.*, **1991**, *49*, 175.
[27] Wong, C.-H.; Halcomb, R.L.; Ichikawa, Y.; Kojimoto, T. *Angew. Chem. Int. Ed. Eng.*, **1995**, *34*, 412.
[28] Machajewski, T.D.; Wong, C.-H. *Angew. Chem. Int. Ed. Eng.*, **2000**, *39*, 1352.
[29] Gijsen, H.J.M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.*, **1996**, *96*, 443.
[30] Merker, R.I.; Troy, F.A. *Glycobiology*, **1990**, *1*, 93.
[31] Blacklow, R.S.; Warren, L. *J. Biol. Chem.*, **1962**, *237*, 3520.
[32] Warren, L.; Blacklow, R.S. *Biochem. Biophys. Res. Comm.*, **1962**, *7*, *433*.
[33] Comb, D.G.; Roseman, S. *J. Biol. Chem.*, **1960**, *235*, 2529.
[34] Brunett, P.; Jourdian, G.W.; Roseman, S. *J. Biol. Chem.*, **1962**, *237*, 2447.
[35] Devries, G.H.; Binkley, S.B. *Arch. Biochem. Biophys.*, **1972**, *151*, 234.
[36] Kim, M.-J.; Hennen, W.J.; Sweers, H.M.; Wong, C.-H. *J. Am. Chem. Soc.*, **1988**, *110*, 6481.
[37] (a) Augé, C.; David, S.; Gautheron, C. *Tetrahedron Lett.*, **1984**, *25*, 4663; (b) Augé, C.; David, S.; Gautheron, C.; Veyrières, A. *Tetrahedron Lett.*, **1985**, *26*, 2439.
[38] Bednarski, M.D.; Chenault, H.K.; Simon, E.S.; Whitesides, G.M. *J. Am. Chem. Soc.*, **1987**, *109*, 1283.
[39] Augé, C.; David, S.; Gautheron, C.; Malleron, A.; Cavayé, B. *New J. Chem.*, **1988**, *12*, 733.
[40] Simon, E.S.; Bednarski, M.D.; Whitesides, G.M. *J. Am. Chem. Soc.*, **1988**, *110*, 7159.
[41] Augé, C.; Bouxom, B.; Cavayé, B.; Gautheron, C. *Tetrahedron Lett.*, **1989**, *30*, 2217.
[42] Liu, J.L.-C.; Shen, G.-J.; Ichikawa, Y.; Rutan, J.F.; Zapata, G.; Vann, W.F.; Wong, C.-H. *J. Am. Chem. Soc.*, **1992**, *114*, 3901.
[43] Gautheron-Le Narvor, C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.*, **1991**, *113*, 7816.
[44] Sparks, M.A.; Williams, K.W.; Lukacs, C.; Schrell, A.; Priebe, G.; Spaltenstein, A.; Whitesides, G.M. *Tetrahedron*, **1993**, *49*, 1.
[45] Lin, C.-H.; Sugai, T.; Halcomb, R.L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.*, **1992**, *114*, 10138.
[46] Kragl, U.; Gygax, D.; Ghisalba, O.; Wandrey, Ch. *Angew. Chem. Int. Ed. Eng.*, **1991**, *30*, 827.
[47] Kragl, U.; Gödde, A.; Wandrey, C.; Lubin, N.; Augé, C. *J. Chem. Soc. PT1*, **1994**, 119.
[48] Augé, C.; Gautheron, C.; David, S. *Tetrahedron*, **1990**, *46*, 201.
[49] Halcomb, R.L.; Fitz, W.; Wong, C.-H. *Tetrahedron: Asymmetry*, **1994**, *5*, 2437.
[50] Kong, D.C.M.; von Itzstein, M. *Tetrahedron Lett.*, **1995**, *36*, 957.
[51] Zhou, P.; Salleh, H.M.; Honek, J.F. *J. Org. Chem.*, **1993**, *58*, 264.
[52] (a) Kuboki, A.; Okazaki, H.; Sugai, T.; Ohta, H. *Tetrahedron*, **1997**, *53*, 2387; (b) Lubineau, A.; Augé, C.; Gautheron-Le Narvor, C.; Ginet, J.C. *BioMed. Chem.*, **1994**, *2*, 669.

[53] Isecke, R.; Brossmer, R. *Tetrahedron*, **1994**, *50*, 7445.
[54] Augé, C.; David, S.; Malleron, A. *Carbohydr. Res.*, **1989**, *188*, 201.
[55] Kragl, U.; Gödde, A.; Wandrey, C. *Tetrahedron: Asymmetry*, **1993**, *4*, 1193.
[56] Augé, C.; Gautheron, C. *J. Chem. Soc., Chem. Commun.*, **1987**, 859.
[57] Schrell, A.; Whitesides, G.M. *Liebigs Ann. Chem.*, **1990**, 1111.
[58] Fitz, W.; Schwark, J.-R.; Wong, C.-H. *J. Org. Chem.*, **1995**, *60*, 3663.
[59] Gijsen, H.J.M.; Wong, C.-H. *J. Am. Chem. Soc.*, **1995**, *117*, 7585.
[60] David, S.; Malleron, A.; Cavayé, B. *New J. Chem.*, **1992**, *16*, 751.
[61] Kok, G.B.; Campbell, M.; Mackey, B.L.; von Itzstein, M. *Carbohydr. Res.*, **2001**, *332*, 133.
[62] Ray, P.H. *Methods Enzymol.*, **1982**, *83*, 525.
[63] (a) Bednarski, M.D.; Crans, D.C.; DiCosimo, R.; Simon, E.S.; Stein, P.D.; Whitesides, G.M. *Tetrahedron Lett.*, **1988**, *29*, 427; (b) Levin, D.H.; Racker, E. *J. Biol. Chem.*, **1959**, *234*, 2532.
[64] Ghalambor, M.A.; Heath, E.C. *Methods Enzymol.*, **1966**, *9*, 534.
[65] Sugai, T.; Shen, G.-J.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.*, **1993**, *115*, 413.
[66] Allen, S.T.; Heintzelman, G.R.; Toone, E.J. *J. Org. Chem.*, **1992**, *57*, 426.
[67] (a) Augé, C.; Delest, V. *Tetrahedron: Asymmetry*, **1993**, *4*, 1165; (b) Augé, C.; Delest, V. *Tetrahedron: Asymmetry*, **1995**, *6*, 863.
[68] Reimer, L.M.; Conley, D.L.; Pompliano, D.L.; Frost, J.W. *J. Am. Chem. Soc.*, **1986**, *108*, 8010.
[69] Draths, K.M.; Pompliano, D.L.; Corley, D.L.; Frost, J.W.; Berry, A.; Disbrow, G.L.; Staversky, R.J.; Lievense, J.C. *J. Am. Chem. Soc.*, **1992**, *114*, 3956.
[70] (a) Sheffer-Dee-Noor, S.; Belakhov, V.; Baasov, T. *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 1583; (b) Coutrot, P.; Dumarcay, S.; Finance, C.; Tabaouyi, M.; Tabaouyi, B.; Grison, C. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 949; (c) Tadanier, J.; Lee, C.; Wittern, D.; Wideburg, N. *Carbohydr. Res.*, **1990**, *201*, 185.
[71] (a) Kaustov, L.; Kababya, S.; Du, S.; Baasov, T.; Gropper, S.; Shoham, Y.; Schmidt, A.; *J. Am. Chem. Soc.*, **2000**, *122*, 2649: (b) Raetz, C.R.H.; Purcell, S.; Meyer, M.V.; Quereshi, N.; Takayama, U. *J. Biol. Chem.*, **1985**, *260*, 16080.
[72] Menger, F.M. *Acc. Chem. Res.*, **1993**, *26*, 206.
[73] (a) Du, S.; Plat, D.; Baasov, T. *Tetrahedron Lett.*, **1996**, *37*, 3545; (b) Du, S.; Plat, D.; Belakhov, V.; Baasov, T. *J. Org. Chem.*, **1997**, *62*, 794.
[74] Cornforth, J.W.; Firth, M.E.; Gatschork, A. *Biochem. J.*, **1958**, *62*, 57.
[75] (a) Williams, D.T.; Perry, M.B. *Canad. J. Biochem.*, **1969**, *47*, 691; (b) Hersberger, Ch.; Davis, M.; Binkley, S.B. *J. Biol. Chem.*, **1968**, *243*, 1585.
[76] Tsvetkov, Y.E.; Shashkov, A.S.; Knirel, Y.A.; Zähringer, U. *Carbohydr. Res.*, **2001**, *335*, 221.
[77] How, M.J.; Halford, M.D.A.; Stacy, M.; Vickers, E. *Carbohydr. Res.*, **1969**, *11*, 313.
[78] Kornilov, V.I.; Bitsherova, I.I.; Turik, S.W.; Zhdanov, Y.A. *Bioorg. Khim.*, **1988**, *14*, 938.

[79] Kornilov, V.I.; Bitsherova, I.I.; Turik, S.W.; Zhdanov, Y.A. *J. Gen. Chem.*, **1987**, *57*, 944.

[80] (a) Mikshier, Y.M.; Paidak, B.B.; Kornilov, V.I.; Zhdanov, Y.A. *J. Gen. Chem.*, **1988**, *68*, 1168; (b) Coutrot, P.; Grison, C.; Tobyaoui, M. *Tetrahedron Lett.*, **1993**, *17*, 5089.

[81] Frost, J.W.; Knowles, J.R. *Biochemistry*, **1984**, *23*, 4465.

[82] (a) Dondoni, A.; Fantin, G.; Fogaguolo, M.; Merino, P. *Tetrahedron Lett.*, **1990**, *31*, 4513; (b) Dondoni, A.; Merino, P. *J. Org. Chem.*, **1991**, *56*, 5294.

[83] (a) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.*, **1968**, 2199; (b) Ahn, N.J. *Top. Curr. Chem.*, **1980**, *88*, 145; (c) Wu, Y.-D.; Houk, N.K. *J. Am. Chem. Soc.*, **1987**, *109*, 908.

[84] Imoto, M.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.*, **1987**, *28*, 6235.

[85] (a) van der Klein, P.A.M.; Boons, G.J.P.H.; Veeneman, G.H.; van der Marel, G.A.; van Boom, J.H. *Synlett*, **1990**, 311; (b) van der Klein, P.A.M.; Boons, G.J.P.H.; Veeneman, G.H.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron Lett.*, **1989**, *30*, 5477.

[86] van der Klein, P.A.M.; de Nooy, A.E.J.; van der Marel, G.A.; van Boom, J.H. *Synthesis*, **1991**, 349.

[87] Bräuer, N.; Kirsching, A.; Schauman, E. *Eur. J. Org. Chem.*, **1998**, 2729.

[88] (a) Corey, E.J.; Erickson, B.W. *J. Org. Chem.*, **1971**, *36*, 3553; (b) Gröbel, B.-T.; Seebach, D. *Synthesis*, **1997**, 357; (c) Tanemura, K.; Dohya, H.; Imamura, M.; Suzuki, T. *Chem. Lett.*, **1994**, 965.

[89] Stork, G.; Zhao, K. *Tetrahedron Lett.*, **1989**, *30*, 287.

[90] Enhsen, A.; Schmidt, R.R. *Liebigs Ann. Chem.*, **1989**, 69.

[91] Tsukamoto, H.; Takahashi, T. *Tetrahedron Lett.*, **1997**, *38*, 6415.

[92] Takahashi, T.; Ksukamoto, H.; Kurosaki, M.; Yamada, H. *Synlett*, **1997**, 1065.

[93] (a) Ramage, R.; Rose, G.W.; MacLeod, A.M. *Tetrahedron Lett.*, **1988**, 4877; (b) Ramage, R.; MacLeod, A.M.; Rose, G.W. *Tetrahedron*, **1991**, *47*, 5625.

[94] (a) Frick, W.; Krülle, T.; Schmidt, R.R. . *Liebigs Ann. Chem.*, **1991**, 435; (b) Krülle, T.; Schmidt, R.R.; Brade, H.; Holst, O. *Carbohydr. Res.*, **1994**, *254*, 141.

[95] (a) Shing, T.K.M. *Tetrahedron*, **1992**, *48*, 6777; (b) Shing, T.K.M. *Tetrahedron: Asymmetry*, **1994**, *12*, 2405.

[96] Dondoni, A.; Marra, A.; Merino, P. *J. Am. Chem. Soc.*, **1994**, *116*, 3324.

[97] Sato, K.-I.; Miyata, T.; Tanai, J.; Yonezawa, Y. *Chem. Lett.*, **1994**, 129.

[98] Itoh, H.; Kaneko, T.; Tanami, K.; Yoda, K. *Bull. Chem. Soc. Jpn.*, **1988**, *61*, 3356.

[99] Khoddir, A.J.; Schmidt, R.R. *Carbohydr. Res.*, **2002**, *337*, 1967.

[100] (a) Lopez-Herrera, F.J.; Sarabia-Garcia, F. *Tetrahedron Lett.*, **1994**, *35*, 6705; (b) Lopez-Herrera, F.J.; Sarabia-Garcia, F. *Tetrahedron*, **1997**, *53*, 3325.

[101] (a) Sarabia, F.; Lopez-Herrera, F.J. *Tetrahedron Lett.*, **2001**, *42*, 8801; (b) Sarabia, F.; Lopez-Herrera, F.J. *Tetrahedron Lett.*, **2001**, *42*, 8805.

[102] Moody, C.J.; Morfitt, C.N. *Synthesis*, **1998**, 1039.

[103] Li, L.-S.; Wu, Y.-L. *Tetrahedron*, **2002**, *58*, 9049.

[104] Liu, K.-G.; Yan, S.; Wu, Y.-L.; Yao, Z.J. *J. Org. Chem.*, **2002**, *67*, 6758.
[105] Liu, K.-G.; Hu, S.-G.; Wu, Y.; Yao, Z.-J.; Wu, Y.-L. *J. Chem. Soc. PT1,* **2002**, 1890.
[106] (a) Branchaud, B.P.; Meier, M.S. *Tetrahedron Lett.*, **1988**, *29*, 3191; (b) Branchaud, B.P.; Meier, M.S. *J. Org. Chem.*, **1989**, *54*, 1320.
[107] Giese, B.; Carboni, B.; Göbel, T.; Muhn, R.; Wetterich, F. *Tetrahedron Lett.*, **1992**, *33*, 2673.
[108] Giese, B.; Linker, T. *Synthesis,* **1992**, 46.
[109] (a) Barton, D.H.R.; Jaszberenyi, J.Cs, Liu, W.; Shinada, T. *Tetrahedron,* **1996**, *52*, 2717; (b) Barton, D.H.R.; Liu, W.; Shinada, T. *Tetrahedron,* **1997**, *53*, 12067; (c) Barton, D.H.R.; Liu, W. *Tetrahedron Lett.*, **1997**, *38*, 367.
[110] Pakulski, Z.; Zamojski,. A. *Tetrahedron,* **1997**, *53*, 3723.
[111] (a) Gordon, D.M.; Whitesides, G.M. *J. Org. Chem.*, **1993**, *58*, 5500; (b) Gordon, D.; Whitesides, G.M. *J. Org. Chem.*, **1993**, *58*, 7937; (c) Choi, S.-K.; Lee, S.; Whitesides, G.M. *J. Org. Chem.*, **1996**, *61*, 7839.
[112] Li, C.J.; Chan, T.H. Organic Reactions in Aqueous Media: John Wiley & Sons, new York, **1977**.
[113] Paquette, L.A.; Mitzel, T.M.; Isaac, M.B.; Crasto, C.F.; Schomer, W.W. *J. Org. Chem.*, **1997**, *62*, 4293.
[114] Warwel, M.; Fessner, W.-D. *Synlett,* **2000**, 865.
[115] (a) Gao, J.; Martinchouk, V.; Whitesides, G.M. *J. Org. Chem.*, **1996**, *61*, 9538; (b) Chan, T.-H.; Xin, Y.-C; von Itzstein, M. *J. Org. Chem.*, **1997**, *62*, 3500.
[116] (a) Reiner, M.; Stolz, F.; Schmidt, R.R. *Eur. J. Org. Chem.*, **2002**, 5760; (b) Reiner, M.; Schmidt, R.R. *Tetrahedron: Asymmetry,* **2000**, *11*, 319.
[117] (a) Barton, D.H.R.; McCombie, U.S. *J. Chem. Soc. PT1,* **1975**, 1574.
[118] (a) Crich, D.; Ritchie, T.J. *J. Chem. Soc. Chem. Commun.*, **1988**, 985; (b) Crich, D.; Ritchie, T.J. *Carbohydr. Res.*, **1989**, C3; (c) Crich, D.; Ritchie, T.J. *J. Chem. Soc. PT1,* **1990**, 945; (d) Paquet, F.; Sinaÿ, P. *J. Am. Chem. Soc.*, **1984**, *106*, 813; (e) Valverde, S.; Garcia-Ochoa, S. *J. Carbohydr. Chem.*, **1989**, *8*, 553.
[119] Buchanan, J.G.; Cleeland, A.P.W.; Johnson, T.; Rennie, R.A.C.; Whightman, R.H. *J. Chem. Soc. PT1,* **1992**, 2593.
[120] (a) Banaszek, A. *Tetrahedron,* **1995**, *51*, 4231; (b) Młynarski, J.; Banaszek, A. *Carbohydr. Res.*, **1996**, *295*, 68; (c) Młynarski, J.; Banaszek, A. *Tetrahedron,* **1997**, *53*, 10643.
[121] Wardrop, D.J.; Zhang, W. *Tetrahedron Lett.*, **2002**, *43*, 5389.
[122] Kumaran, G.; Mootao, D.R. *Tetrahedron Lett.*, **2001**, *42*, 3783.
[123] Houdrechy, A.; Sinaÿ, P. *J. Org. Chem.*, **1992**, *57*, 4142.
[124] Lay, L.; Nicotra, F.; Panza, L.; Russo, G.; Sello, G. *J. Carbohydr. Chem.*, **1998**, *17*, 1269.
[125] (a) Crich, D.; Hwang, J.-T.; Yuan, H. *J. Org. Chem.*, **1996**, *61*, 6189; (b) Dondoni, A.; Scherrmann, M.-C. *J. Org. Chem.*, **1994**, *59*, 6404. (c) Hengewald, J.E.; Grief, V.; Tadanier, J.; Lee, C.-M.; Riley, D.; Lartey, P.A.

*Tetrahedron Lett.*, **1984**, *25*, 4075; (d) Horton, D.; Issa, M.; Priebe, W.; Scheidman, M.L. *Carbohydr. Res.*, **1993**, *246*, 105.

[126] Yates, P.; Krepinsky, J.J.; Seif-el-nasr, A. *J. Chem. Soc. Chem. Commun.*, **1989**, 177.

[127] (a) Boeckman, Jr., R.K.; Bruza, K.J. *Tetrahedron,* **1981**, *23*, 3997; (b) Friesen, R.W.; Sturino, C.F.; Daljeet, A.K.; Kolaczewska, A. *J. Org. Chem.*, **1991**, *56*, 1944; (c) Barnes, M.P.; Probert, R.H.; Weightman, R.H. *J. Chem. Soc. PT1,* **1995**, 431.

[128] Jiang, S.; Rycroft, A.D.; Singh, G.; Wang, X.-Z.; Wu, L. *Tetrahedron Lett.*, **1998**, *39*, 3809.

[129] Kolb, M.; *Synthesis*, **1990**, 171.

[130] (a) Mlynarski, J.; Banaszek, A. *Tetrahedron Lett.*, **1998**, *39*, 5425: (b) Mlynarski, J.; Banaszek, A. *Tetrahedron,* **1999**, *55*, 2785; (c) Mlynarski, J.; Banaszek, A. *Polish J. Chem.*, **1999**, *73*, 973.

[131] Mikołajczyk, M.; Graczyk, P.P.; Wieczorek, M.W. *J. Org. Chem.*, **1994**, *59*, 1672.

[132] (a) Rollin, P.; Sinaÿ, P. *Carbohydr. Res.*, **1981**, *98*, 139; (b) De Lederkramer, R.M.; Varela, O. *Adv. Carbohydr. Chem. Biochem.*, **1992**, *50*, 125.

[133] Młynarski, J.; Banaszek, A. *Org. Lett.*, **1999**, *1*, 1709.

[134] Młynarski, J.; Banaszek, A. *Tetrahedron: Asymmetry,* **2000**, *11*, 3737.

[135] Danishefsky, S.J.; DeNinno, M.P.; Chen, S. *J. Am. Chem. Soc.*, **1988**, *110*, 3929.

[136] Banwell, M.; De Savi, Ch.; Watson, K. *J. Chem. Soc. PT1,* **1998**, 2251.

[137] Nugent, T.C.; Hudlicky, T. *J. Org. Chem.*, **1998**, *63*, 510.

[138] Banwell, M.; De Savi, Ch.; Watson, K. *Chem. Commun.*, **1998**, 1189.

[139] Hudlicky, T.; Mandel, M.; Rauden, J.; Lee, R.S.; Bachmann, B.; Dudding, T.; Yost, K.J.; Merola, J.S. *J. Chem. Soc. PT1,* **1994**, 1553.

[140] Burke, S.D.; Voight, E.A. *Org. Lett.*, **2001**, *3*, 237.

[141] Voight, E.A.; Rein, Ch.; Burke, S.D. *Tetrahedron Lett.*, **2001**, *42*, 8747.

[142] Ray, P.H.; Benedict, C.D.; Grasmuk, H. *J. Bacteriol.*, **1981**, *145*, 1273.

[143] Burke, S.D.; Sametz, G.M. *Org. Lett.*, **1999**, *1*, 71.

[144] Lubineau, A.; Augé, J.; Lubin, N. *Tetrahedron,* **1993**, *49*, 4639.

[145] Lubineau, A.; Queneau, Y. *J. Carbohydr. Chem.*, **1995**, *14*, 1295.

[146] Lubineau, A.; Arcostanzo, H.; Queneau, Y. *J. Carbohydr. Chem.*, **1995**, *14*, 1307.

[147] Hu, Y.-J.; Huang, Y.D.; Yao, Z.-J.; Wu, Y.-L. *J. Org. Chem.*, **1998**, *63*, 2456.

[148] Li, L.-S.; Wu, Y.L.; Wu, Y. *Org. Lett.*, **2000**, *2*, 891.

[149] Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron,* **1987**, *43*, 5909.

[150] Martin, S.F.; Zinke, P.W. *J. Org. Chem.*, **1991**, *56*, 6600.

[151] Craig, D.; Pennington, M.W.; Warner, P. *Tetrahedron Lett.*, **1995**, *36*, 5815.

[152] Smith, D.B.; Wang, Z.; Schreiber, S.L. *Tetrahedron ,* **1990**, *46*, 4793.

[153] Schlessinger, R.H.; Pettus, L.H. *J. Org. Chem.*, **1998**, *63*, 9089.

[154] Turner, N.J.; Whitesides, G.M. *J. Am. Chem. Soc.*, **1989**, *111*, 624.

[155] Devianne, G.; Escudier, J.-M.; Baltas, M.; Gorrichon, L. *J. Org. Chem.*, **1995**, *60*, 7343.
[156] Ruland, Y.; Zedde, C.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.*, **1999**, *40*, 7323.
[157] Sugisaki, C.H.; Ruland, Y.; Le Clezio, I.; Baltas, M. *Synlett*, **2001**, 1905.
[158] Johnson, C.R.; Kozak, J. *J. Org. Chem.*, **1994**, *59*, 2910.

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# STRUCTURE-ACTIVITY RELATIONSHIPS OF NATURALLY OCCURRING ACTIVE FORMS OF VITAMIN D ANALOGUES

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**ABSTRACT:** We synthesized active forms of naturally occurring vitamin D analogues (vitamin $D_2$, $D_3$, $D_4$, $D_5$, $D_6$ and $D_7$) and investigated the biological activities and metabolism (especially for 1α,25-dihydroxyvitamin $D_4$). 1α,25-Dihydroxyvitamin $D_4$ showed the same affinity for vitamin D receptor as 1α,25-dihydroxyvitamin $D_3$, but it had stronger affinity for vitamin D binding protein and more efficient biological activities, such as activation of vitamin D receptor and effect on cellular growth and differentiation than 1α,25-dihydroxyvitamin $D_3$. 1α,25-Dihydroxyvitamin $D_4$ increased total bone mass content in animal models of osteoporosis without elevating plasma calcium level. The pharmacokinetic study on 1α,25-dihydroxyvitamin $D_4$ showed longer $T_{1/2}$, higher $C_{max}$ and AUC values than those of 1α,25-dihydroxyvitamin $D_3$. According to the metabolism study, 1α,25-dihydroxyvitamin $D_4$ is metabolized in a similar manner to 1α,25-dihydroxyvitamin $D_2$ but differently from 1α,25-dihydroxyvitamin $D_3$, suggesting the importance of theside chain structure. The results indicate that 1α,25-dihydroxyvitamin $D_4$ is a promising therapeutic agent for osteoporosis, skin disease and immune disorders. The synthetic procedure developed for active forms of vitamin D analogues was applied to the synthesis of biologically active steroids such as dictyosterol possessing the neurite outgrowth activity and the intermediates in steroid biosynthesis.

## INTRODUCTION

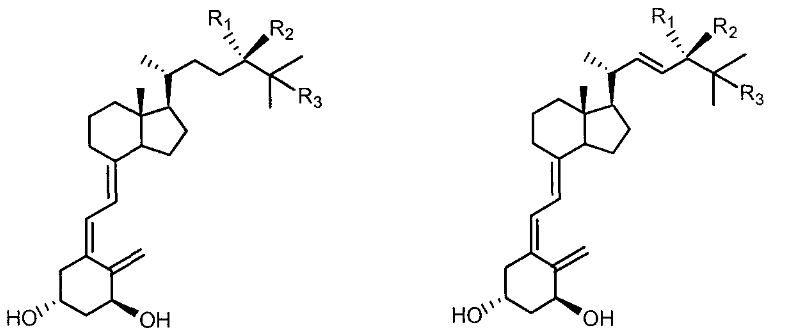
Much attention hasbeen paid to the natural products becausethey have not only various biological activities but also are possible ligands for nuclear receptors, which are ligand-responsible transcription factors. Naturally occurring vitamin D analogues (vitamin $D_2$, $D_3$, $D_4$, $D_5$, $D_6$ and $D_7$) exhibit biological activities after converting to active metabolites, which act on a nuclear receptor, vitamin D receptor (VDR).

Active forms of vitamin $D_3$ [1], namely 1α,25-dihydroxyvitamin $D_3$1α,25$(OH)_2D_3$ and its derivatives have been widely studied for their versatile biological activities. They regulate calcium metabolism in intestine, kidney and bone, maturation and differentiation of normal and malignant cells and immune responsiveness [2-4]. The finding that 1α,25 $(OH)_2D_3$ serves as a potent modifier for multifunctional biological responses in many tissues and cells [5] implies its promising application to therapy for cancers [6], psoriasis [7,8], and immune disorders [9]. The clinical usage of 1α,25 $(OH)_2D_3$ is , however, limited by the accompanying calcemic activity [10]. For this reason, analogues of 1α,25$(OH)_2D_3$ have been synthesized with side chain modifications [11].

It has been demonstrated that the relative potencies of the rachitic bone healing of vitamin$D_2$ [12], vitamin $D_3$, vitamin $D_4$ (a 24S methyl derivative of $D_3$) [13], vitamin $D_5$ (a 24R ethyl derivative of $D_3$) [14], vitamin $D_6$ (a derivative with a 24S-ethyl and a double bond between C-22/C-23 of vitamin $D_3$) [15] and vitamin $D_7$ (a 24R methyl derivative of vitamin $D_3$) [16] are 100, 100, 75, 2, 1 or 10, respectively [17]. The vitamin D analogues must be hydroxylated at C-25 position and be followed by 1α-hydroxylation to exhibit the biological activities. The in vivo activities of vitamin D analogues are influenced by many factors, such as metabolic conversion (activation and deactivation) and pharmacokinetiic properties. The side chain structure of vitamin D analogues will affects their metabolic transformation and result in reducing biological activities because of increased clearance. For example, vitamin $D_2$ has a different side chain skelton from vitamin $D_3$ but they are equally able to show same biological activities such as stimulation of intestinal calcium transport, induction of bone calcium mobilization, elevation of serum phosphorous and healing of rickets in mammal. However, in regard to biological activities, vitamin $D_2$ is 1/5 to 1/10 as active as vitamin $D_3$ in birds [18,19]. Therefore the difference between the antirachitic activity of vitamin D analogues reported previously may not be the direct effects of their active metabolites. Thus it is interesting to compare the biological activities of naturally occurring vitamin D analogues with those of their corresponding active forms. The substitution of a R or a S methyl ($D_4$ and $D_7$) or a ethyl group at C-24 position ($D_5$), double bond between C-22/C-23 or a combination of both

($D_2$ and $D_6$) on $1\alpha(OH)_2D_3$ and $1\alpha,25(OH)_2D_3$ may induce remarkable changes in the biological activities and the analysis of structure–activity relationships among these compounds should be important to develop vitamin D analogues for clinical use . The chemical structures of active forms of vitamin D analogues are illustrated in Fig. (**1**).

We report here the synthesis, biological activities and metabolism of the active forms of naturally occurring vitamin D analogues, especially active form of vitamin $D_4$ ($1\alpha,25(OH)_2D_4$), in comparison with those of $1\alpha,25(OH)_2D_3$. In addition, we introduce application of the synthetic procedure developed for the preparation of active forms of vitamin D analogues described here to the synthesis of biologically active natural steroids and the intermediates in ergosterol biosynthesis.




**1**: $R_1=R_2=H$, $R_3=OH$, $1\alpha,25(OH)_2D_3$
**2**: $R_1=CH_3$, $R_2=H$, $R_3=OH$, $1\alpha,25(OH)_2D_4$
**3**: $R_1=H$, $R_2=C_2H_5$, $R_3=OH$, $1\alpha,25(OH)_2D_5$
**4**: $R_1=H$, $R_2=CH_3$, $R_3=OH$, $1\alpha,25(OH)_2D_7$
**5**: $R_1=R_2=R_3=H$, $1\alpha(OH)D_3$
**6**: $R_1=CH_3$, $R_2=R_3=H$, $1\alpha(OH)D_4$
**7**: $R_1=R_3=H$, $R_2=C_2H_5$, $1\alpha(OH)D_5$
**8**: $R_1=R_3=H$, $R_2=CH_3$, $1\alpha(OH)D_7$

**9**: $R_1=CH_3$, $R_2=H$, $R_3=OH$, $1\alpha,25(OH)_2D_2$
**10**: $R_1=H$, $R_2=CH_3$, $R_3=OH$, 24-epi-$1\alpha,25(OH)_2D_2$
**11**: $R_1=H$, $R_2=C_2H_5$, $R_3=OH$, $1\alpha,25(OH)_2D_6$
**12**: $R_1=CH_3$, $R_2=R_3=H$, $1\alpha(OH)D_2$
**13**: $R_1=R_3=H$, $R_2=CH_3$, 24-epi-$1\alpha(OH)D_2$
**14**: $R_1=R_3=H$, $R_2=C_2H_5$, $1\alpha(OH)D_6$

**Fig. (1).** Chemical structures of 1 α,25-dihydroxyvitamin D and 1α-hydroxyvitamin D analogues

## SYNTHESIS OF ACTIVE FORMS OF VITAMIN D ANALOGUES

The active forms of vitamin D analogues (**1-14**) were synthesized by the coupling of the steroid moiety [20] with the corresponding phenyl sulfone derivatives [21] to constitute the side chain part followed by the UV irradiation of the provitamins and the subsequent thermal isomerization of the previtamin D derivatives [22]. These active forms of vitamin D analogues were also prepared through the cyclovitamin D derivatives [23].

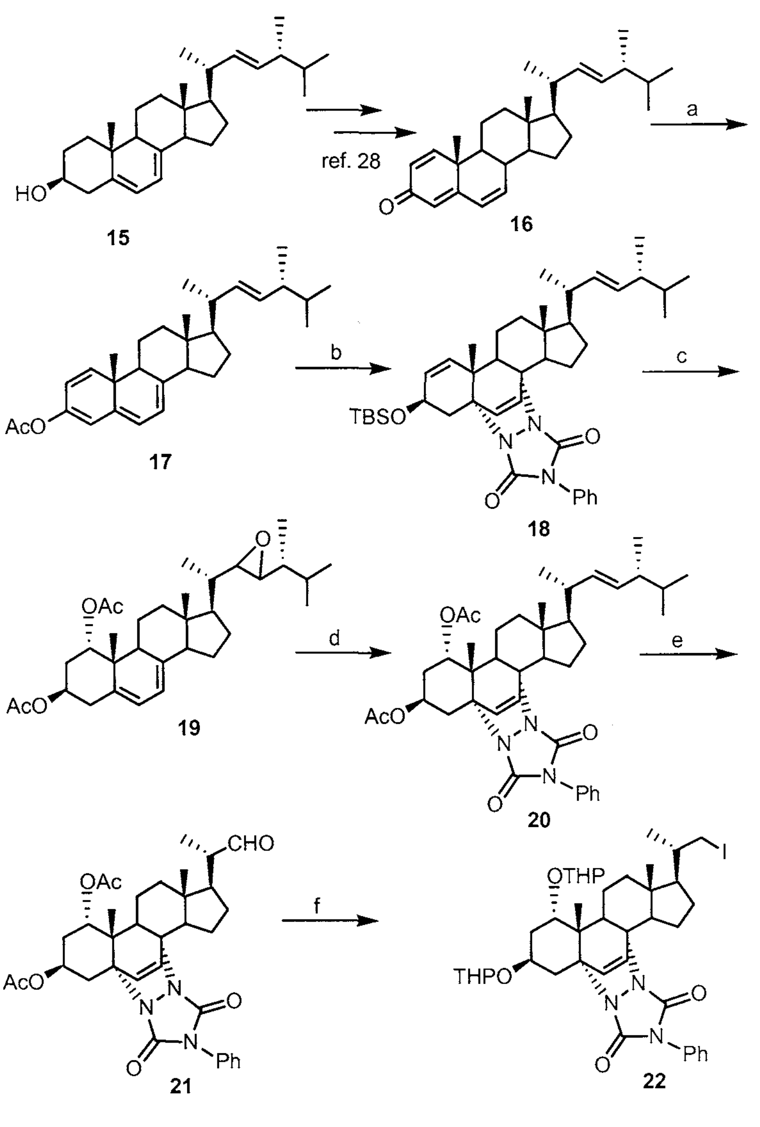
### Synthesis of steroid moiety

Enolacetylation of the trienon (**16**) [24] derived from ergosterol (**15**) gave the tetraenyl acetate (**17**) [25]. Reduction of **17** with $Ca(BH_4)_2$, protection of the hydroxyl group as t-butyldimethylsilyl (TBS) ether and the 5,7-dien with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) afforded **18**. Epoxidation of the double bond of **18** with m-CPBA, $LiAlH_4$ reduction, acetylation, deoxygenation of **19** with $NaI/(CF_3CO)_2O$ and reprotection of the 5,7-diene with PTAD provided **20**. Ozonolysis of **20**, reduction of **21** with $NaBH_4$, tosylation and subsequent iodonization with NaI produced the C-22 iodide derivative (**22**) (Scheme (**1**)) [26].

### Synthesis of phenyl sulfone derivatives

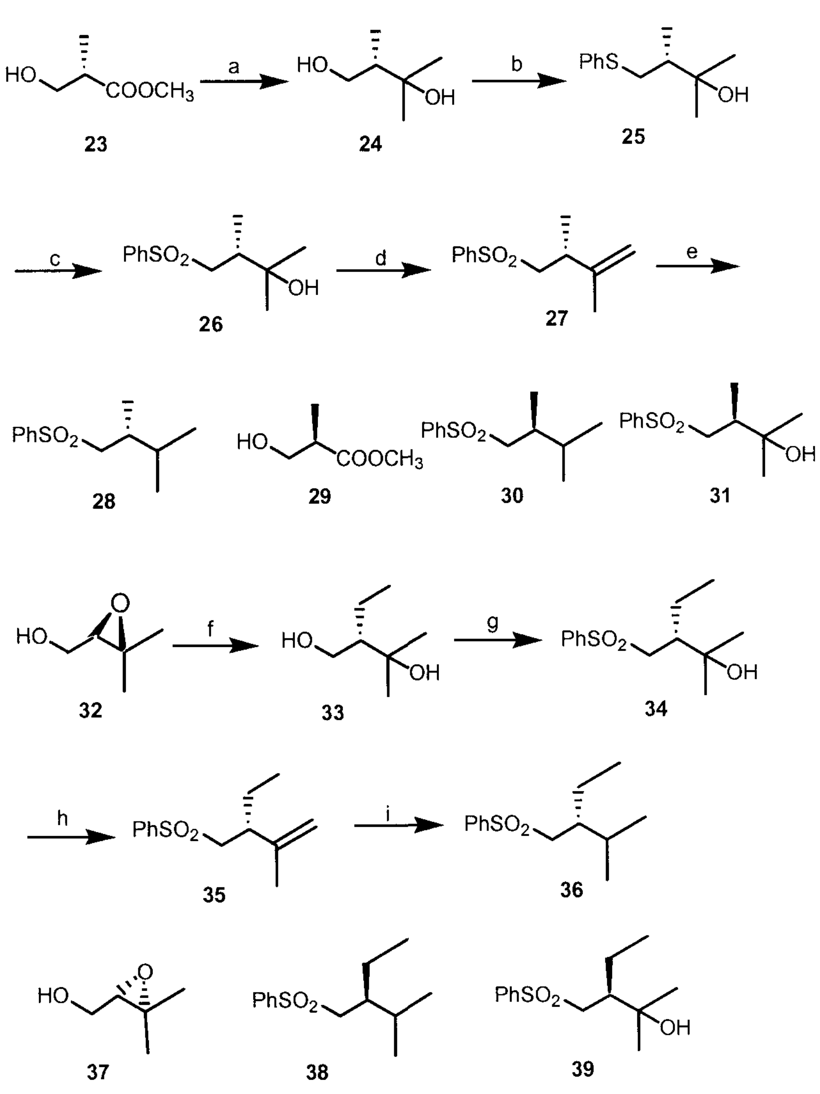
Treatment of the hydroxybutylate (**23**) with MeMgI gave the diol (**24**). Tosylation of **24**, treatment with thiophenol and oxidation with m-CPBA of **25** gave the phenyl sulfone derivative (**26**). Selective dehydration of **26** with MsCl-$Et_3N$ and hydrogenation gave the phenyl sulfone derivative (**28**). Cleavage of the optically active epoxy ring of **32** with $C_2H_5MgBr$ in the presence of CuI afforded the diol (**33**), which was treated in a similar manner as described above to give the phenyl sulfone derivatives (**36**). Treatment of the antipodes (**29** and **37**) produced the corresponding phenyl sulfone derivatives **30**, **31**, **38** and **39** respectively (Scheme (**2**)).

**Scheme (1).** Synthesis of steroid moiety




Reagents. a) Isopropenyl acetate, p-TsOH; b) (i) $Ca(BH_4)_2$, (ii) PTAD, (iii) TBSCl, imidazole, 69%; c) (i) *m*-CPBA, (ii) *n*-$Bu_4NF$, (iii) $LiAlH_4$, (iv) $Ac_2O$/Py, 51%; d) (i) $(CF_3CO)_2O$, NaI, 40%; (ii) PTAD, 96% ; e) $O_3$, $Me_2S$; f) (i) KOH/EtOH, (ii) DHP, PPTS, (iii) $NaBH_4$, 71%, (iv) TsCl/Py, (v) NaI, 73%.

**Scheme (2).** Synthesis of phnyl sulfone derivatives




Reagents. a) (i) DHP, p-TsOH, (ii) MeMgI, (iii) p-TsOH/MeOH, 84%; b) (i) TsCl/Py, (ii) PhSH, t-BuOK, 94%; c) m-CPBA, 85%; d) MsCl, $Et_3N$, 81%; e) $PtO_2/H_2$, 75%; f) $C_2H_5MgBr$, CuI, 62%; g) (i) TsCl/Py, (ii) PhSH, t-BuOK, (iii) m-CPBA, 82%; h) MsCl, $Et_3N$, 94%; i) $PtO_2/H_2$, 80%.

The phenyl sulfone derivatives having larger alkyl groups such as propyl and butyl groups were also synthesized in a similar manner. The phenyl sulfone derivatives described here are employed for the preparation of various types of natural steroids derivatives [27]. This will be reported later.

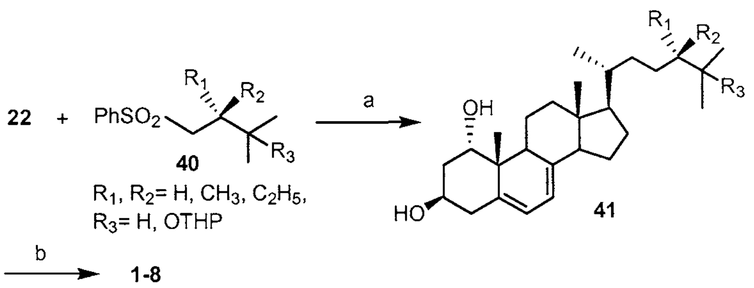
## Synthesis of active forms of vitamin D analogues

Condensation of C-22 iodide derivative (**22**) with the phenyl sulfone derivatives of tetrahydropyranyl (THP) ether of **40** in the presence of n-BuLi and 2,3-dimethyl-2-imidazolinone (DMI), removal of the THP and PTAD groups [28] and reductive elimination of phenylsulfonyl group with 5% Na-Hg afforded the provitamin D derivatives (**41**). UV irradiation (high pressure mercury lamp) of **41** and subsequent thermal isomerization gave the active forms of vitamin D analogues (**1-8**) (Scheme (**3**)) [29]. Coupling of C-20 aldehyde derivative (**21**) with phenyl sulfone derivative (**40**) gave provitamin D derivatives (**42**) with double bond between C-22/C-23. Similar treatment of **42** as described above afforded the active forms of vitamin D analogues (**9-14**) (Scheme (**3**) and (**4**)) [30].

Ozonolysis of the PTAD adduct of ergosterol (**43**) gave the C-20 aldehyde (**44**). Similar treatment of **44** as described for **21** gave **45**. Condensation of **45** with phenyl sulfone derivatives (**40**), regeneration of the 5,7-diene, deprotection of the hydroxyl group and reductive elimination of phenyl sulfonyl group afforded **46**. UV irradiation and thermal isomerization of **46** gave vitamin D derivatives (**47**). Tosylation of **47** followed by the treatment with base in methanol gave 3,5-cyclovitamin D derivatives (**48**). Oxidation of **48** with t-BuOOH/$SeO_2$, acetolysis (**49**) and subsequent saponification produced the active forms of vitamin D analogues (**1-8**). Similar treatment of C-20 aldehyde (**44**) yielded the active forms of vitamin D analogues with double bond between C-22/C-23 (**9-14**) (Scheme (**5**)).

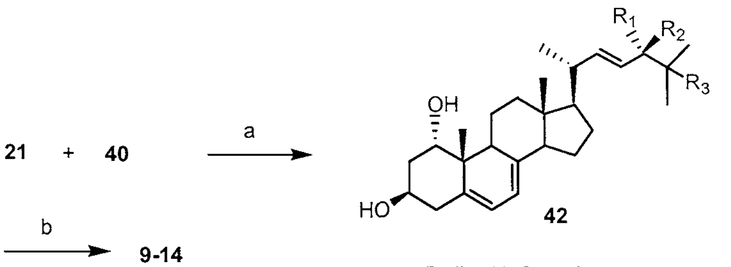
The compound (**18**) derived from **15** [31] was the good synthetic intermediate for the preparation of the 2β-hydroxylated active forms of vitamin D derivative such as ED-71[32]. Epoxidation of **50** derived from the ozonolysis of **18** followed

**Scheme (3).** Synthesis of active forms of vitamin $D_3$, $D_4$, $D_5$ and $D_7$




Reagent. a) (i) n-BuLi, DMI, (ii) p-TsOH/MeOH, (iii) 5% Na-Hg, (iv) $LiAlH_4$, b) UV, heat.

**Scheme (4).** Synthesis of active forms of vitamin $D_2$, 24-epi-$D_2$ and $D_6$

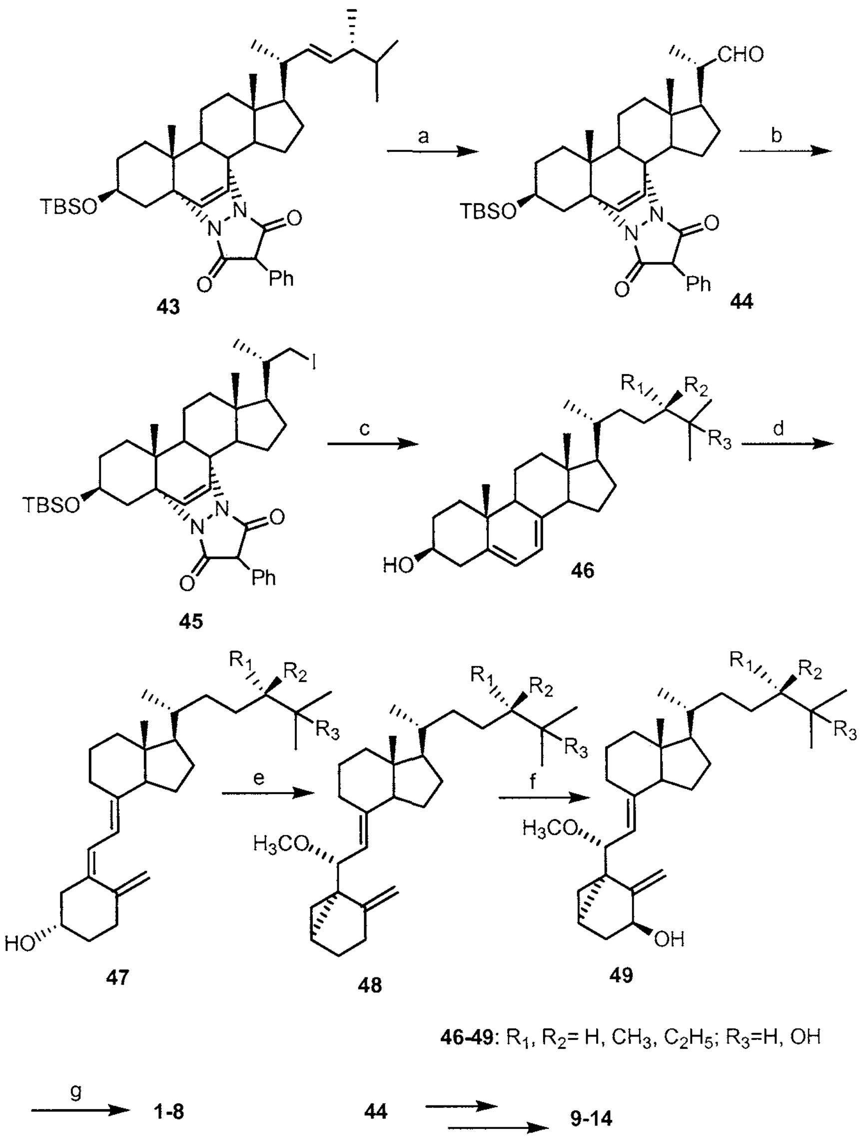



**41, 42** : $R_1$,$R_2$=H, $CH_3$, $C_2H_5$, $R_3$=H, OH

Reagents. a) (i) n-BuLi, (ii) p-TsOH/MeOH, (iii) 5% Na-Hg, (iv) $LiAlH_4$, b) UV, heat.

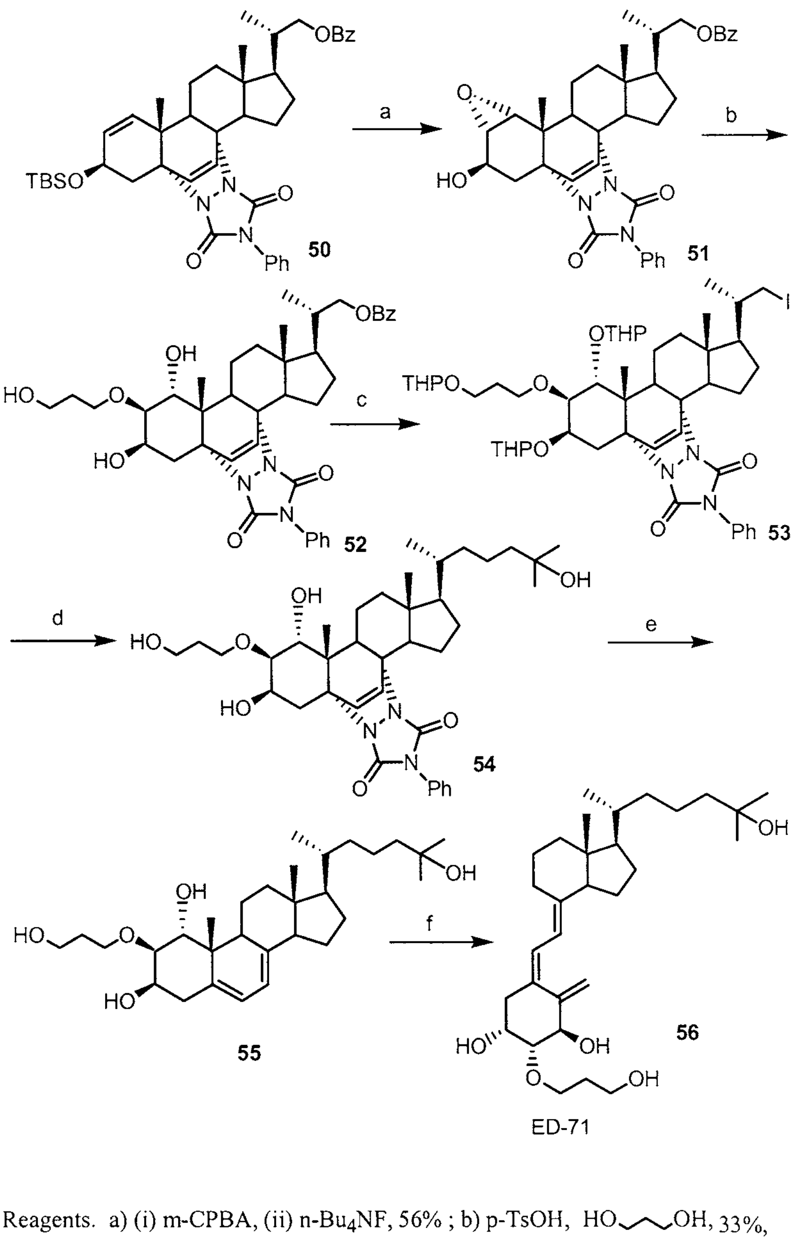
by the ring opening of the 1α,2α-epoxide of **51** with 1,3-propanediol under acidic condition afforded **52**. Protection of the hydroxyl groups as THP ethers and the subsequent construction of the side chain unit, deprotection of the hydroxyl groups and 5,7-diene group provided **55**, which was treated in a usual manner (UV irradiation and thermal isomerization) to perform ED-71 (**56**) (Scheme (**6**)).

**Scheme (5).** Synthesis of active forms of vitamin D derivatives (via cyclovitamin D derivatives)



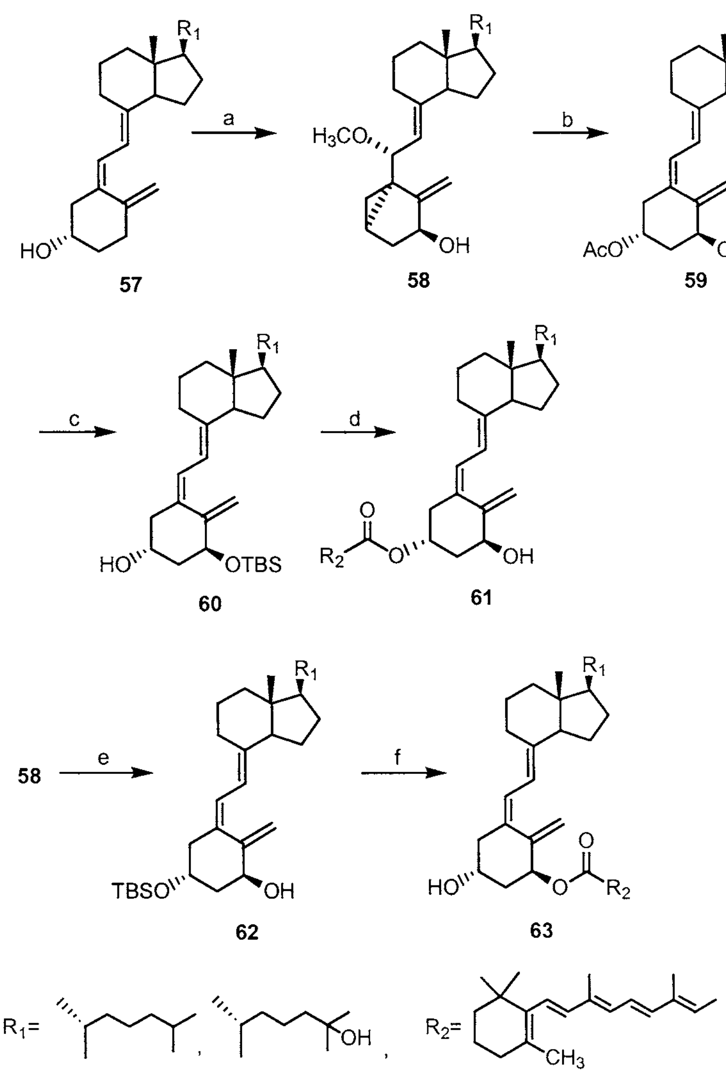

Reagents. a) $O_3$, $Me_2S$; b) (i) $NaBH_4$, (ii) TsCl/Py, NaI; c) (i) n-BuLi, DMI, **40**, (ii) n-$Bu_4NF$, (iii) 5% Na-Hg, (iv) DBU; d) (i) UV, (ii) heat; e) (i) TsCl/Py; (ii) $NaHCO_3$/MeOH; f) t-BuOOH, $SeO_2$; g) (i) AcOH, (ii) KOH/EtOH.

**Scheme (6).** Synthesis of ED-71




Reagents. a) (i) m-CPBA, (ii) n-$Bu_4NF$, 56% ; b) p-TsOH, HO⌒⌒OH, 33%, c) (i) DHP, PPTS, (ii) KOH/EtOH, (iii)TsCl/Py, (iv) NaI, 60 % ; d) (i) n-BuLi, DMI, $PhSO_2$⌒⌒OTHP ; (ii) p-TsOH/MeOH, (iii) 5% Na-Hg ; e) $LiAlH_4$, 68% ; UV, heat, 12%.

**Scheme (7).** Synthesis of the ester of retinoic acid and active forms of vitamin $D_3$.




Reagents. a) (i) $NaHCO_3$/MeOH, (ii) t-BuOOH, $SeO_2$ ; b) (i) AcOH, (ii) t-$BuMe_2SiCl$, imidazole ; c) KOH/EtOH ; d) (i) $R_2COOH$, $(CF_3CO)_2O$, (ii) n-$Bu_4NF$ ; e) (i) $Ac_2O$/Py, (ii) p-TsOH, $H_2O$, (iii) t-$BuMe_2SiCl$, imidazole, (iv) KOH/EtOH ; f) (i) $R_2COOH$, $(CF_3CO)_2O$, (ii) n-$Bu_4NF$.

## BIOLOGICAL ACTIVITY

Okano et al. assessed the structure-activity relationships of the active forms of vitamin D analogues especially with respect to the structural differences in the configuration of the methyl group at C-24 position and the role of a double bond between C-22/C-23 in the side chain of 1α,25$(OH)_2$D analogues [33].

### Binding assay for calf-thymus vitamin D receptor

The binding affinity of 1α,25$(OH)_2D_2$ and 1α,25$(OH)_2D_4$ for VDR was almost the same as that of 1α,25$(OH)_2D_3$, while 24-epi-1α,25$(OH)_2D_2$ and 1α,25$(OH)_2D_7$ bound to VDR about 80% and 60% less effectively than 1α,25$(OH)_2D_3$. It implies that the introduction of a 24S methyl group or the combination of a 24S methyl group and the double bond at C-22/C-23 in the side chain of 1α,25$(OH)_2D_3$ does not affect VDR binding affinity. On the other hand, the introduction of a 24R methyl group or a combination of a 24R-methyl group and the double bond at C-22/C-23 reduces VDR binding affinity significantly. Accordingly, a 24S-methyl group, not a 24R-methyl group, may be preferred for VDR binding and therefore for biological activity.

### Binding assay for rat serum vitamin D-binding protein (DBP)

Except for 24-epi-1α,25$(OH)_2D_2$, the binding affinity of the 1α,25$(OH)_2$D analogues for rat plasma DBP was much higher than that of 1α,25$(OH)_2D_3$. 24-Epi-1α,25$(OH)_2D_2$ exhibited 50% less binding affinity for DBP compared with 1α,25$(OH)_2D_3$. It implies that the introduction of a 24S-methyl group or a 24R-methyl group into the side chain of 1α,25$(OH)_2D_3$ increases DBP binding affinity significantly. The introduction of a 24S-methyl group in the presence of C-22/C-23 double bond in the side chain increases the affinity while the addition of 24R-methyl group decreased it. It appears that a C-22/C-23 double bond may serve to reduce DBP binding. The binding affinities of ED-71 (**56**) to chick embryonic intestinal receptor and rat plasma DBP were

approximately 8 times weaker and twice stronger than the respective values of 1α,25$(OH)_2D_3$ [34].

## Assessment of cell proliferation and differentiation

It has been shown that the ability of 1α,25$(OH)_2D_3$ metabolites and their analogues to inhibit proliferation of HL-60 cells is almost proportional to their ability to induce differentiation of the cells [35]. We also observed the same tendency for the derivatives tested. The antiproliferative and differentiation-inducing effect of 1α,25$(OH)_2D_3$ and 1α,25$(OH)_2D_4$ were nearly proportional to their binding affinity for VDR. In contrast, despite the comparable ability to bind to VDR, the antiproliferative and differentiation-inducing effect of 1α,25$(OH)_2D_2$ were slightly lower than that of 1α,25$(OH)_2D_3$ and 1α,25$(OH)_2D_4$. Furthermore, despite the lower binding affinity for VDR, 24-epi-1α,25$(OH)_2D_2$ had a comparable ability to suppress cell proliferation and 70% of the ability of that of 1α,25$(OH)_2D_3$ to induce cell differentiation of HL-60 cells. The analogue, 1α,25$(OH)_2D_7$ was the least potent derivative tested although it exhibited a higher binding affinity for VDR than 24-epi-1α,25$(OH)_2D_2$.

## Bone resorbing activity

The bone resorbing activities of 1α,25$(OH)_2D_2$ and 1α,25$(OH)_2D_4$ were almost the same as for 1α,25$(OH)_2D_3$. It is positively related to binding affinity to VDR. The results clearly explain that the calcemic effects of 1α,25$(OH)_2D_2$ and 1α,25$(OH)_2D_4$ are comparable to that of 1α,25$(OH)_2D_3$ as expected from their binding affinity for VDR. Sato et al. reported that bone calcium mobilization of 24-epi-1α,25$(OH)_2D_2$ and 1α,25$(OH)_2D_7$ in vitamin D deficient rats was about 50% of that by 1α,25$(OH)_2D_3$ by assessing serum calcium changes after intravenous administration [36]. The results of biological activities of 1α,25-$(OH)_2D$ analogues were shown in Table 1 in comparison with those of 1,25$(OH)_2D_3$.

**Table 1. Biological activities of 1α,25(OH)$_2$D analogues (% of 1α,25(OH)$_2$D$_3$ effect)**

| Biological activities | 1α,25(OH)$_2$D Analogues | | | |
|---|---|---|---|---|
| | 1α,25(OH)$_2$D$_2$ | (24-epi-) 1α,25(OH)$_2$D$_2$ | 1α,25(OH)$_2$D$_4$ | 1α,25(OH)$_2$D$_7$ |
| Competitive binding to 1,25(OH)$_2$D$_3$ calf-thymus receptor | 100 | 20 | 100 | 30 |
| Competitive binding to vitamin deficient rat serum DBP | 240 | 40 | 500 | 1280 |
| Differentiation of human HL-60 leukemia cells | 60 | 70 | 100 | 40 |
| Inhibition of proliferation of human HL-60 leukemia cells | 80 | 100 | 110 | 60 |

## Transcriptional activity

Biological activities of 1α,25(OH)$_2$D$_3$ are predominantly expressed through VDR, which regulate the transcription of target genes after 1α,25(OH)$_2$D$_3$ binding to VDR [37]. VDR is a member of the super family of nuclear receptors [38,39], such as thyloid hormone, retinoic acid, glucocorticoids and estrogen [40,41]. The transfection experiments on MG-63 cells indicate that the activities of 1α,25(OH)$_2$D$_2$ and 1α,25(OH)$_2$D$_4$ to activate VDR on reporter having two vitamin D response elements (VDREs) from promoter of rat 25-hydroxyvitamin D$_3$-24-hydroxylasae gene were significantly higher (about 170%) than that of 1α,25(OH)$_2$D$_3$, whereas 24-epi-1α,25(OH)$_2$D$_2$ and 1α,25(OH)$_2$D$_7$ had about 70% and 20% of the effect of 1α,25(OH)$_2$D$_3$. The vitamin D analogues had similar effects on a VDRE of human osteocalcin gene promoter in MG-63 cells [42]. The results correlate well with their binding affinity for VDR (Table 2).

Table 2. Transcriptional activities of 1α ,25(OH)$_2$D analogues on various cells (% of 1α ,25(OH)$_2$D$_3$ effect)

| Biological activities | 1α,25(OH)$_2$D Analogues | | | |
|---|---|---|---|---|
| | 1α,25(OH)$_2$D$_2$ | ( 24-epi-) 1α,25(OH)$_2$D$_2$ | 1α,25(OH)$_2$D$_4$ | 1α,25(OH)$_2$D$_7$ |
| Human osteosarcoma MG-63 cells[1)] | 170 | 70 | 170 | 20 |
| Human osteosarcoma MG-63 cells[2)] | 125 | 80 | 115 | 25 |

1) MG-63-cells cotransfected with a luciferase reporter plasmid (pGVB2 vector) inserted with a rat 25-hydroxyvitamin D$_3$-24-hydroxylase gene promotor (-291/+3) including two VDREs as an internal control.

2) MG-63-cells cotransfected with a luciferase reporter plasmid (pGVB2 vector) inserted with a human osteocalcin gene promotor (-848/+10 ) including a VDREs and a pRL-CMV as an internal control .

## Assay for intestinal calcium transport and in vivo bone mobilization

The activities of 1α,25(OH)$_2$D$_4$ on a calcium transport and bone mobilization in vitamin D deficient SD rats were less than (about 1/2) those of 1α,25(OH)$_2$D$_3$. This is well understood by stronger affinity of 1α,25(OH)$_2$D$_4$ for DBP than that of 1α,25(OH)$_2$D$_3$ because of the decreased availability for target cells (decreased uptakes into the cells)[43,44]. The hypercalcemic activities of 24-epi-1α,25(OH)$_2$D$_2$ and 1α,25(OH)$_2$D$_7$ were negligible as compared with that of 1α,25(OH)$_2$D$_3$. Their effects on intestinal calcium transport was significantly smaller than that of 1α,25(OH)$_2$D$_3$ and it is required 10 fold more of these compounds to produce a similar activity to 1α,25(OH)$_2$D$_3$ [35].

## Enhancement of growth inhibition and differentiation in human monoblastic leukemia cells by combination with hydroxyurea

**Table 3. Growth inhibition in human monoblastic leukemia U937 cell by vitamin D analogues in combination with hydroxyurea (HU).**

| Compounds | Growth (% of control) | | |
|---|---|---|---|
| | -HU | + HU | Ratio[a)] |
| None | 100 | 54 | 100 |
| 1α ,25$(OH)_2D_3$ (3 x $10^{-9}$ M) | 74 | 25 | 46 |
| 24-epi-1α ,25$(OH)_2D_2$ (9 x $10^{-9}$ M) | 87 | 33 | 61 |
| 1α ,25$(OH)_2D_7$ (9 x $10^{-9}$ M) | 89 | 32 | 59 |
| 1α $(OH)D_3$ (3 x $10^{-8}$ M) | 72 | 27 | 50 |
| 1α $(OH)D_2$ (6 x $10^{-8}$) | 91 | 31 | 57 |
| 1α $(OH)D_4$ (6 x $10^{-9}$M) | 89 | 28 | 52 |
| 1α $(OH)D_7$ (6 x $10^{-9}$ M) | 100 | 27 | 50 |

Cells (5 x $10^4$ cells $ml^{-1}$) were cultuted with vitamin D analogues in the absence or presence of 50 mM HU for 4 days.

a) Ratio (%) represents the growth of cells in combination with that of cells treated with 50 mM HU alone.

1α,25-Dihydroxyvitamin $D_3$ is a potent inducer of differentiation in myeloid leukemia cells, but its clinical use is limited because of its hypercalcemic activity. To overcome this adverse effect of vitamin D, many trials have been carried out. Makishima et al. reported that 1α,25$(OH)_2$D and 1α$(OH)_2$D analogues showed effective synergism with hydroxyurea (HU) with regard to growth inhibition of human monoblastic leukemia U937 cells (Table 3) [45]. The combination of active forms of vitamin D analogues with HU may be useful in the chemo-differentiation therapy of acute monocytic leukemia. It is known that retinoids in combination with active forms of vitamin D derivatives show synergistic effect in inducing the cellular differentiation and growth inhibition. Expecting synergistic effect we synthesized the ester of active form of vitamin $D_3$ and retinoic acid. Acetolysis of **58** and protection of **59** as a 1α-TBS ether followed by the saponification of 3β-acetyl group gave **60**.

Treatment of **60** with retinoic acid in the presence of $(CF_3CO)_2O$ afforded **61**. Acetylation of 1α-hydroxyl group of **58**, hydrolysis and protection of 3β-hydroxyl group as a TBS ether afforded **62**, which was treated in a similar manner as described above furnished **63** (Scheme (**7**)). The biological activities will be reported elsewhere.

### Effect on animal models with osteoporosis

Considering the stronger binding affinity for DBP over 1α,25$(OH)_2D_3$, 1α, 25$(OH)_2D_4$ is expected to function as a mild and long active compound in vivo. We studied the effect of 1α, 25$(OH)_2D_4$ on the animal model with osteoporosis. To the animal model with osteoporosis (retired 7-10 months old Fischer rats) [46], an adequate dose of 1α,25$(OH)_2D_3$, 1α,25$(OH)_2D_4$ and 1α$(OH)D_3$ were administered to the models orally 5 times a week and for further three months. The rats were sacrificed and the total bone mineral content (BMC) and volume (BMV) were measured. Preliminary study showed that 1α,25$(OH)_2D_4$ was more effective on the BMC and BMV at the doses that exhibit only minimal effects on plasma concentration compared with 1α,25$(OH)_2D_3$ and 1α$(OH)D_3$. This finding should be at least partly according to the longer stability of 1α,25$(OH)_2D_4$ in circulation because of stronger affinity for DBP compared with 1α,25$(OH)_2D_3$.

## METABOLISM STUDY OF 1α,25$(OH)_2D_4$

As described above, 1α,25$(OH)_2D_4$ showed biological activities equal to those of 1α,25$(OH)_2D_3$ but also have a five times greater binding affinity for DBP. Its biological activities in vivo may be due to the direct binding to the receptor and be influenced by the factors described before, such as metabolism and pharmacokinetics. Among these factors, the interactions with DBP are particularly important. It has been shown that biological properties and extra cellular pharmacokinetics of 1α,25$(OH)_2D$ analogues can be largely predicted from their affinity for DBP. In general, vitamin D analogues with high affinity for DBP are more stable in circulation than

those with low affinity [47,48]. This suggests that 1α,25$(OH)_2D_4$will be a long active vitamin D analogue in place of 1α,25$(OH)_2D_3$. In this chapter, we describe the pharmacokinetics and metabolic pathway based on the study of metabolites of 1α,25$(OH)_2D_4$ in comparison with those of 1α,25$(OH)_2D_3$ [49].

**Table 4. Pharmacokinetics parameters of 1α,25$(OH)_2D_3$ and 1α,25$(OH)_2D_4$ after a single oral administration in male rat**

| Pharmacokinetics parameters | 1α,25$(OH)_2$D Analogues | |
|---|---|---|
| | 1α,25$(OH)_2D_3$ | 1α,25$(OH)_2D_4$ |
| *T*max (hr) | 2.0 | 2.0 |
| Cmax (pg/ml) | 661.9 | 757.5 |
| $T_{1/2}$ (2-12) (hr) | 3.5 | 5.9 |
| *AUC* (0-48) (pg.hr/ml) | 7197.5 | 11611.7 |
| *AUC* (0-48)-BG (pg.hr/ml) | 3241.2 | 10517.3 |

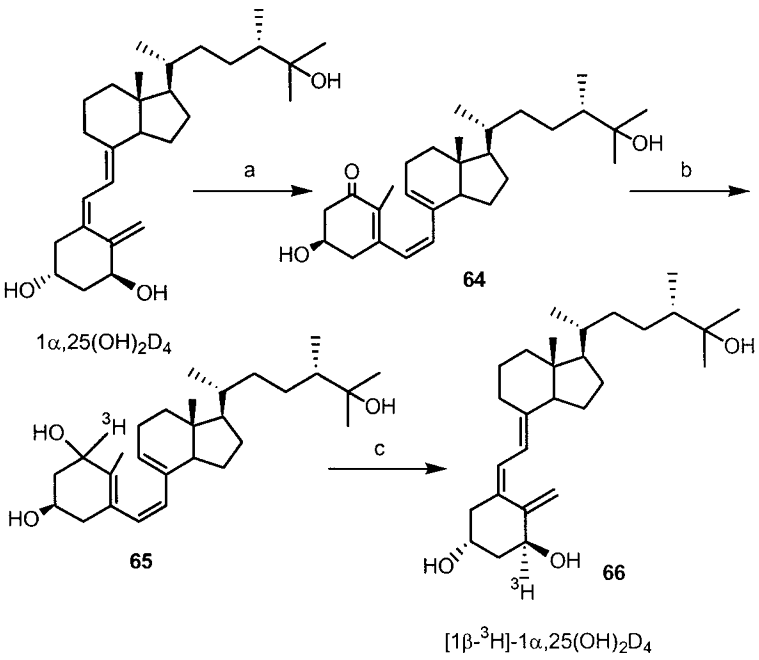
## Pharmacokinetics study

In order to clarify the pharmacological difference between 1α,25$(OH)_2D_3$ and 1α,25$(OH)_2D_4$, we examined the time course of blood concentrations after oral administration of these drugs to rats and dogs by a radio-receptor assay (RRA). Honma et al. found that the plasma concentration of 1α,25(OH)2$D_4$ showed longer $T_{1/2}$, higher $C_{max}$ and AUC values than those of 1α,25$(OH)_2D_3$. The results are well explained by the stronger affinity of 1α,25$(OH)_2D_4$ for DBP than that of 1α,25$(OH)_2D_3$ (Table 4 and Table5).

**Table 5. Pharmacokinetics parameters of 1α ,25(OH)$_2$D$_3$ and 1α ,25(OH)$_2$D$_4$ after a single oral administration in male dog**

| Pharmacokinetics parameters | 1α,25(OH)$_2$D Analogues | |
|---|---|---|
| | 1α,25(OH)$_2$D$_3$ | 1α,25(OH)$_2$D$_4$ |
| *T*max (hr) | 2.0 | 3.0 |
| *C*max (pg/ml) | 248.0 | 421.5 |
| $T_{1/2}$ (2-12) (hr) | 8.1 | 8.1 |
| *AUC* (0-48) (pg.hr/ml) | 3062.5 | 4595.0 |
| *AUC* (0-48)-BG (pg.hr/ml) | 2153.7 | 4423.1 |

**Scheme (8).** Synthesis of [1β-$^3$H]-1α,25(OH)$_2$D$_4$.



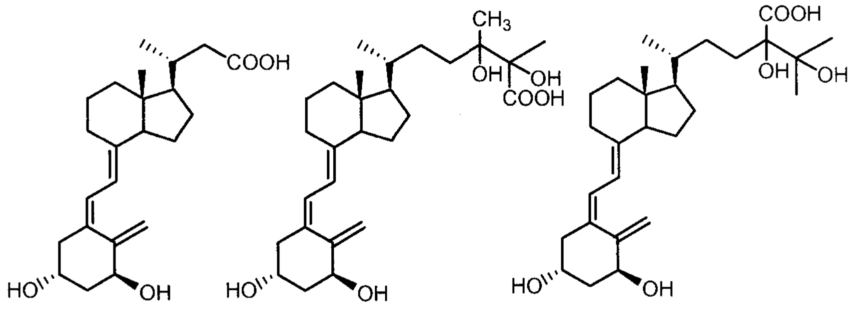

Reagents. a) $MnO_2$; b) $NaB^3H_4$; c) heat, HPLC, 0.3%.

## Metabolites and metabolic pathway

It is well established that the main metabolic pathway of 1α,25(OH)2$D_3$ is initiated with hydroxylation at C-24 position followed by formation of 24-keto derivative and hydroxylation at C-23 position and terminated at formation of calcitric acid as a final product. In order to examine the effect of the methyl group at the side chain on the metabolism and elucidate the metabolic pathway of 1α,25$(OH)_2D_4$, tritium labeled 1α,25$(OH)_2D_4$ was synthesized from 1α,25$(OH)_2D_4$ in three steps (oxidation of hydroxyl group at C-1 position, reduction with $NaB^3H_4$ and thermal isomerization) as shown in Scheme (**8**). The ethanol solution of 1α,25$(OH)_2D_4$ and a trace amount of [1β-$^3$H]-1α,25$(OH)_2D_4$ were orally administered to Wister rats. The bile from the rats was collected and extracted [50]. The extract after methylation with diazomethane was purified by Sephadex LH-20 chromatography, by reversed phase and normal phase HPLC and three main metabolites were detected (**M1**, **M2** and **M3**) in a ratio of 1:1:1 (Figure (**2**)). The metabolite (**M1**) was found to be calcitroic acid by comparing the spectral data with those reported in the literature [51]. The structures of the other two metabolites (**M2** and **M3**) were determined by spectral analyses, $^{13}$C-NMR (DEPT, HMQC), $^1$H-NMR and mass spectra, and $NaIO_4$ cleavage of the proposed diol structure of the methyl esters (**M2** and **M3**)(stereochemistry of the metabolites at the around C-24 and C-25 is not determined at this stage )(Figure (**3**)).In the case of 1α,25$(OH)_2$ $D_4$, it is difficult to form corresponding 24-keto derivative due to the methyl group at C-24 position in contrast to 1α,25$(OH)_2D_3$. This may be the main reason why 1α,25$(OH)_2D_4$ is metabolized to give **M2** and **M3** together with **M1**. The affinity of the methyl esters of **M2** and **M3** for VDR was 1/100 and 3/100 compared with that of 1α,25$(OH)_2D_3$. Antiproferative ability on cells were almost proportional to the affinity for VDR (1/100 and 1/10 respectively) compared with that of 1α,25$(OH)_2D_3$. The binding affinity of the esters for DBP was very low probably because of the steric hindrance to the hydroxyl group at C-25 position.

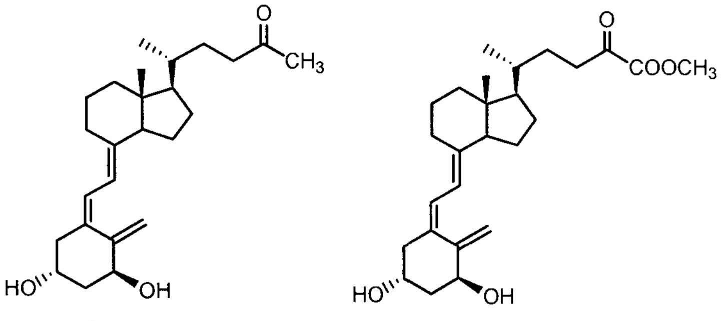
Taking the results into consideration, 1α,25 $(OH)_2D_4$ is metabolized in a similar way to that of 1α,25$(OH)_2D_2$ in spite of the absence of the double bond between C-22/C-23 in the side chain but differently from

1α,25$(OH)_2D_3$ due to the methyl group at C-24 position. The proposed metabolic pathway is shown in Scheme (**9**) [52,53]. It is concluded that the long acting 1α,25$(OH)_2D_4$ is a promising drug for the treatment of osteoporosis, immune disorders and skin diseases with higher stability and lower toxicity than 1α,25$(OH)_2D_3$.




**M1** **M2** **M3**

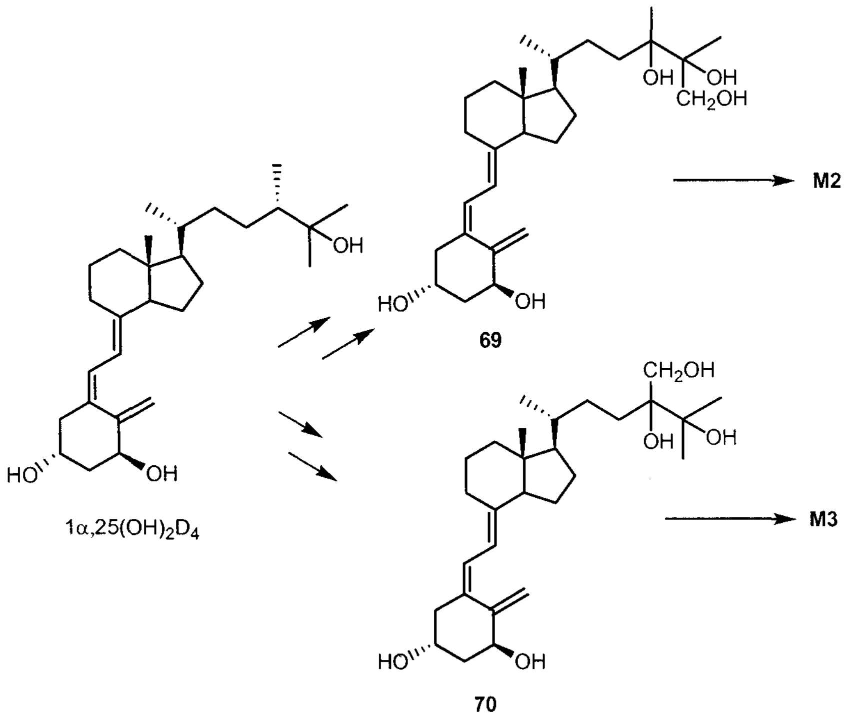
**Fig. (2).** Chemical structures of metabolites (**M1, M2, M3**)




**67** **68**

**Fig. (3).** $NaIO_4$ oxidation products of **M2** and **M3** (methyl ester)

**Scheme (9).** Proposed metabolic pathway of 1α,25$(OH)_2D_4$ in rat




## STEREOSELECTIVE SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL STEROIDS

The synthetic procedure developed for the preparation of active forms of vitamin D analogues was applied to the synthesis of various natural steroids by the reaction of steroid moieties with phenyl sulfone derivatives described here.

Neurotropic factors (NTFs) are known to be essential for the survival and functional maintenance of nerve cells [54]. Decreased availability of NTFs is thought to cause various nerve disorders including Altzheimer's disease [55]. For example, nerve growth factor (NGF) was found as a prototypical neurotrophin factor essential for growth and development of neurons in the central as well as peripheral nervous system [56].

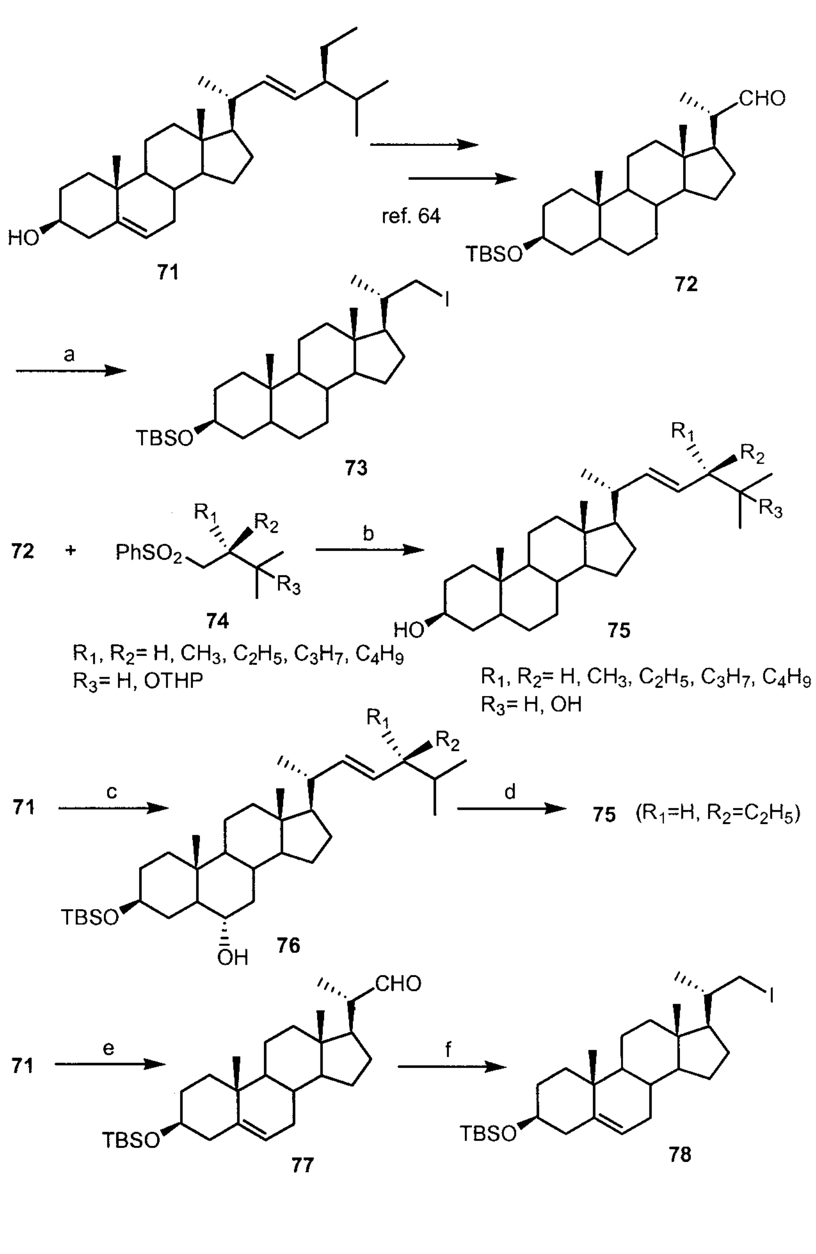
However, it is very difficult to use NGF as a therapeutic agent since it must be administered intraventricularly. NGF is a large molecule that is impermeable across the blood-brain barrier. Therefore, much efforts have been focussed on finding promising therapeutic drugs in natural compounds with low molecular weight, such as microbial metabolites [57-60] which elicit or enhance neurotrophic actions and prevent neuronal cell death. In the course of screening of natural product having the neurite outgrowth activity in the neurons of the rat hypothalmas [61], Kawahara et al. isolated an active compound from the culture broth of the cellular slime mold *Dictyosterlium purpureum* K1001. They determined the structure of the compound as poriferast-22-en-3β-ol (dictyosterol, **75**: $R_1=C_2H_5$, $R_2=H$ ) [62,63] by means of $^1H$- and $^{13}C$-NMR spectra. However, the yield of dictyosterol obtained from the culture broth was very low. To study the biological activities in detail, it is necessary to provide a sufficient amount of sample.

Sucraw et al. synthesized dictyosterol but their procedure required the isolation of the desired enantiomers from the diastereomeric mixtures [64]. We report here the first stereoselective synthesis of dictyosterol and the related compounds possessing the neurite outgrowth activity in the hypothalamic neurons of rat by the coupling of steroid derivative (**72** or **73**) with pheny sulfone derivatives (**74**) to constitute the side chain segment. The 24-epimer (**75**: $R_1=H$, $R_2=C_2H_5$) of the dictyosterol was alternatively prepared in 6 steps starting from stigmasterol (**71**) (Scheme (**10**)). Dictyosterol and its 24-epimer showed the same activity indicating that the configuration of the ethyl group at C-24 position is not important in the neurite outgrowth activity. The activity of poriferastanol was very low (20% of astrocyte-conditioned medium (ACM) effect) compared with that of dictyosterol (76% of ACM effect). Although the natural steroids such as campesterol and sitosterol showed no activity, ergosterol and stigmasterol was active on the neurite outgrowth. 3β-Acetate derivative of dictyosterol was inactive. These results indicate that the presence of the double bond between C-22/C-23 and hydroxyl group (free) arerequired for the neurite outgrowth activity. In connection with the effect of the alkyl groups at the C-24 position, the biological activity was in the order, $C_2H_5>CH_3>H$. However, the derivatives having larger alkyl groups such as propyl or butyl groups at C-24 position showed no activity [65].

The supply of the authentic sample is important in the study of biosynthesis. We synthesized the intermediates of biosynthesis of ergosterol from lanosterol in yeast [66]. Treatment of methyl phenyl sulfone (**79**) with epoxy alcohol (**80**) afforded phenyl sulfone derivative (**83**) [67]. Coupling of **83** with C-22-iodide derivative (**47**), transformation of the coupling product (**84**) into 28-iodide derivative (**85**) followed by the elimination of hydrogen iodide and PTAD in the presence of DBU furnished the biosynthetic intermediate (**86**). The treatment of C-20 aldehyde derivative (**44**) in place of **47** provided the intermediate (**88**) (Scheme (**11**)). The ergosterol derivatives obtained here are also important compounds for biosynthetic study such as 22,23-dihydroergosterol (provitamin $D_4$) and 24-epi-22,23-dihydroergosterol (provitamin $D_7$).

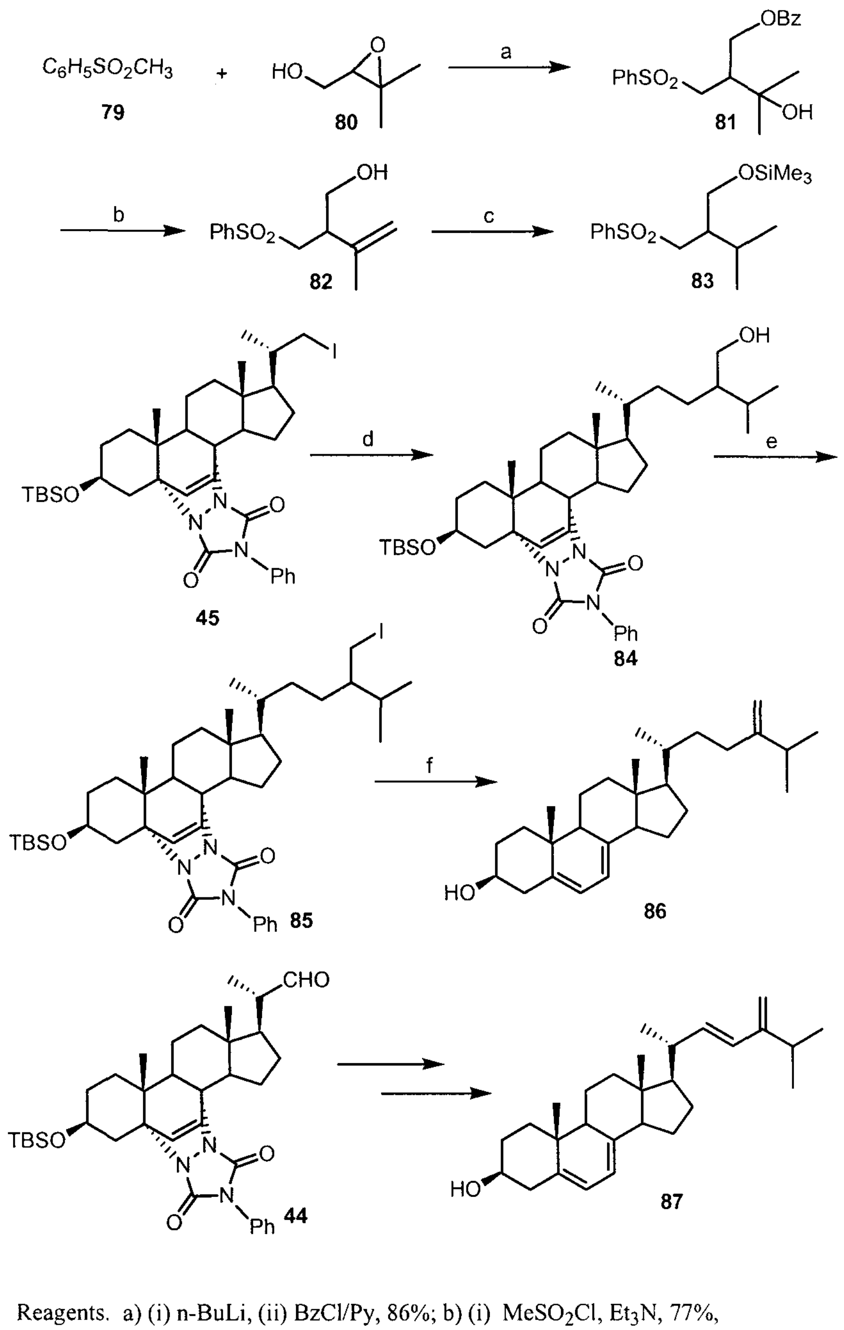
In order to study the biological activity of the metabolites (**M2** and **M3**) in detail, we examined to synthesize them. The similar derivative (**89**) possessing the diene structure in the side chain seemed to be the good candidate as the synthetic intermediate. Thus the coupling of the C-22 iodide derivative (**45,** t-butyldiphenylsilyl ether) and trimethylsilyl ether of **82** gave **88**, which was converted to **89** in a similar manner as described above. The compound (**89**) was treated with m-CPBA followed by the reduction with $LiALH_4$ to afford **90** as a less polar product and **91** as a more polar product in a ratio of 3:2. The respective derivative was converted to the compound (**92** and **93**) in seven steps (epoxidation with m-CPBA, hydrolysis with 0.1N $H_2SO_4$, oxidation with Py□$SO_3$ followed by the oxidation with $NaClO_2$, methylation with trimehtylsilyl diazomethane and the elimination of the protecting group of the 5,7-diene and the hydroxyl groups) (Scheme (**12**)). The conversion of **92** and **93** to **M2** and **M3** is now underway. Furthermore, the procedure on steroid moieties and phenyl sulfone derivatives described here was able to apply for the preparation of conventional natural steroids as shown in Fig. (**4**).

**Scheme (10).** Synthesis of dictyosterol and related derivatives



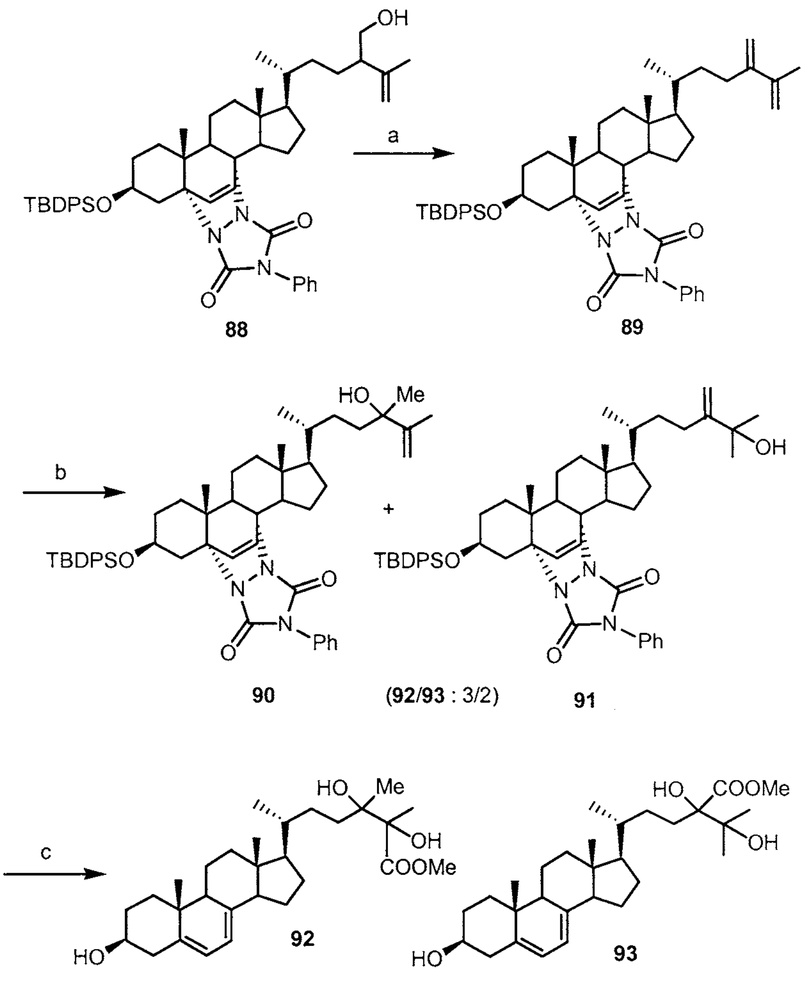

Reagents. a) (i) TsCl/Py, (ii) NaI, 75% ; b) (i) LDA, (ii) 5% Na-Hg, (iii) p-TsOH/MeOH; c) (i) TBSCl, imidazole, (ii) $BH_3$-$Me_2S$, (iii) $H_2O_2$, NaOH, 52% ; d) (i) TsCl/Py, (ii) $LiAlH_4$, (iii) p-TsOH/MeOH, 72% ; e) $O_3$, $Me_2S$, 77% ; f) (i) TsCl/Py, (ii) NaI, 86%.

**Scheme (11).** Synthesis of biosynthetic intermediates of ergosterol




Reagents. a) (i) n-BuLi, (ii) BzCl/Py, 86%; b) (i) $MeSO_2Cl$, $Et_3N$, 77%, (*ii*) KOH/EtOH, 75%; c) (*i*) $H_2$/Pd-C, 100%, (*ii*) $Me_3SiCl$, imidazole, 75%; d) (i) n-BuLi, DMI, **83**, (ii) 5% Na-Hg, $Na_2HPO_4$, 70%; e) (i) TsCl/Py, (ii) NaI, 86%; f) (i) DBU, 61%, (ii) n-$Bu_4NF$, 86%.

**Scheme (12).** Synthesis of the intermediates of the metabolites (**M2** and **M3**)




Reagents. a) (i) p-TsCl/Py, (ii) NaI, (iii) DBU (reflux in benzene), 74%, ; (b) (i) m-CPBA, (ii) $LiAlH_4$, PTAD, 44%, ; (c) (i) m-CPBA, (ii) 0.1 N $H_2SO_4$, 53%, ; (iii) Py-$SO_3$, $Et_3N$/DMSO, (iv) $NaClO_2$, $NaH_2PO_4$, 2-methyl-2- butene, (v) $Me_3SiCHN_2$/MeOH, 61%, ; (vi) DBU (reflux in xylene), 85%, ; (vii) n-$Bu_4NF$/THF-MeOH, 44%.

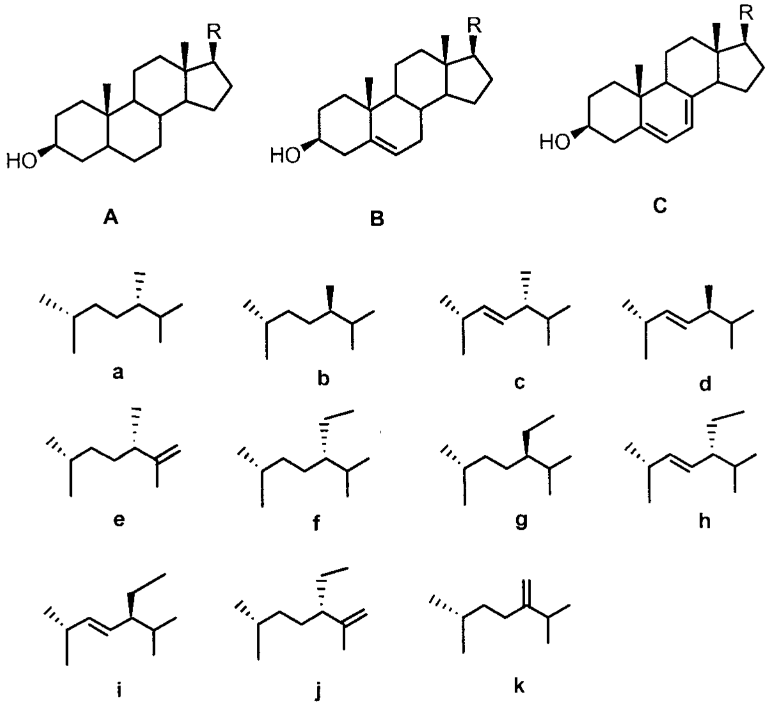



**Fig (4).** Structures of conventional steroids

A-a:ergostanol; A-b: campestanol; A-c: brassicasterol; A-f: poriferastanol; A-g: stigamastanol; A-k: 24-methylcholestanol; B-a: dihydrobrassicasterol; B-b: campesterol; B-d: clinosterol; B-e: codisterol; B-f: clionosterol: B-g: sitosterol; B-h: poriferasterol; B-j: clerosterol; B-k: chalinasterol; C-a: 22-dihydroergosterol; C-b: 7-dehydrocampesterol; C-f : 7-dehydroclionosterol; C-g: 7-dehydrositosterol; C-i: 7-dehydrostigmasterol.

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## REFERENCES


[1] Holick, M. F.; Schnoes, H. K.; DeLuca, H.F.; Suda, T.; Cousins, R.,; *J. Biochemistry,* **1971,** 10, 2799-2804.

[2] Minghetti, P. P.; Norman, A.W.; *FASEB J.*, **1988**, 2, 3043-3053.

[3] Walters, M. R.; *Endocr. Rev.,* **1992**□13, 719-764.

[4] Abe, E.,; Miyaura, C.; Sakagami, H.; Takeda, M.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T.; *Proc. Natl. Acad. Sci. USA*., **1981**, 78, 4990-4994.

[5] Honma, Y.; Hozumi, M.; Abe, E.; Konno, K.; Fukushima, M.; Hata, S.; Nishii,Y.; DeLuca, H. F.; Suda, T.; *Proc. Natl. Acad. Sci. U.S.A*., **1983**, 80, 201-204.

[6] Palmieri, G. M.; *J. Clin. Endocrin. Metab*., **1997**, 82, 3516-3517.

[7] Bikle, D. D.; *Endocri. Rev*., **1992**, 13, 765-784.

[8] Reichrath, J.; Perez, A.; Muller, S..M.; Chen, T. C.; Kerber, A.; Bahmer, F. A.; Holick, M.F.; *Acta .Derm. Nenereol*., **1997**, 77, 268-272.

[9] Snyman, J.R.; De Sommers, K.M.; Steinmann, M.A.; Lizamore, D. J.; *Eur. J. Clin. Pharmacol*., **1997**, 52, 277-280.

[10] Bishop, J. E.; Collins, E.D.; Okamura, W. H.; Norman, A.W.; *J. Bone Miner. Res*., **1994**, 9, 1277-1288.

[11] Bouillon, R.; Okamura, W. H.; *Endocr. Rev*., **1995**, 16, 200-257.

[12] Grab, W.; *Hoppe-Seylers Z. Physiol. Chem*., **1936**, 241, 63-89.

[13] Windaus, A.; Trautmann, G.; *Hoppe-Seylers Z. Physiol. Chem*., **1937**, 247, 185-188.

[14] Wunderlich, W.; *Hoppe-Seylers Z .Physiol. Chem*., **1936**, 241, 116-124.

[15] Linsert, O.; *Hoppe-Seylers Z. Physiol. Chem*., **1936**, 241, 125-128.

[16] Ruigh, W. L.; *J. Am. Chem. Soc*., **1942**, 64, 1900-1902.

[17] DeLuca, H. F.; Weller, M.; Blunt, J. W.; Neville P. F.; *Arch. Biochem. Biophys*., **1968**, 124, 122-124.

[18] Sjorden, G.; Smith ,C; Lindgren, U, DeLuca, H.F.; Proc. Soc. Exp. Biol. Med., **1985**, 178, 432-436.

[19] Ikekawa, N.; *Med., Res. Rev*., **1987**, 7, 333-366.

[20] Tachibana, Y.; *Nippon Kagaku Kaishi,* **1992**, 53-62.

[21] Tachibana, Y.; Yokoyama, S.; Tsuji, M.; *Bull. Chem. Soc. Jpn*., **1989**, 62, 2579-2603.

[22] Tachibana, Y.; Tsuji, M. In *Studies in Natural Products Chemistry*; Attaur-ur-Rahman, Ed., Elsevier Science B.V: Amsterdam, **1993**: Vol. 11, pp. 379-408.

[23] Tachibana, Y.; Yokoyama, S.; Tejima, T.; *Nippon Kagaku Kaishi*, **1994**, 345-353.

[24] Pelc, B.; Kodicek, E.; *J. Chem. Soc., C*, **1971**, 859-860.

[25] Tachibana, Y.; *Bull. Chem. Soc. Jpn.*, **1986**, 59, 3702-3704.

[26] Tachibana, Y.; *Bull. Chem. Sco. Jpn.*, **1988**, 61, 3915-3918.

[27] Stonik, V. A.; Ponomareniko, T. N.; Makarieva, V. M.; Boguslavsky, A.S.; Dmitrenok, S. N.; Fedorov, S. N.; Strobikin, S.A.; Compt.Biochem. Physiol., Part. B, **1998**, 120, 337-347.

[28] Tachibana, Y.; *Chem. Pharm. Bull.*, **1998**, 46, 1454-1458.

[29] Tsuji, M.; Yokoyama, S.; Tejima, T.; Tachibana, Y.; Ikekawa, N.; *Bull. Chem. Soc. Jpn.*, **1990**, 63, 2233-2238.

[30] Tsuji, M.; Yokoyama, S.; Tejima, T.; Tachibana,Y.; *Bull. Chem. Soc. Jpn.*, **1989**, 62, 3132-3137.

[31] Tachibana,Y.; *Nippon Kagaku Kaishi*, **1996**, 91-96.

[32] Miyamoto, K.; Maruyama, E.; Ochi, K.; Watanabe, H.; Kubodera, N.; *Chem. Pharm. Bull.*, **1993**, 41, 1111-1113.

[33] Tachibana, Y.; Tsuji, M.; *J. Synth. Org. Chem. Jpn.*, **1995**, 53, 65-75.

[34] Okano, T.; Tsugawa, N.; Masuda, S.; Takeuchi, A.; Kobayashi, T.; Takita, Y.; Nishii, Y.; *Biochem. Biophys. Res. Commun.*, **1989**, **163**, 1444-1447.

[35] Miyaura, C.; Abe, E.; Kuribayashi, T.; Tanaka, H.; Konno, K.; Nishii, Y.; Suda, T.; *Biochem. Biophys. Res. Commun.*, **1981**, 102, 937-943.

[36] Ostern, V. K.; Lau, W. F.; Lee, S.; Perlmann, K.; Prahl, J.; Schnoes, H. K.; DeLuca, H. F.; Ikekawa, N.; *J. Biol. Chem.*, **1987**, 262, 14164-14171.

[37] Pike, J. W.; *Annu. Rev. Nutr.*, **1991**, 11, 189-216.

[38] Evans, R. M.; *Science*, **1988**, 240, 889-895.

[39] Umesono, K.; Murakami, K. K.; Thompson, C. C.; Evans, R. M.; *Cell*, **1991**, 65, 1255-1266.

[40] Kliewer, S. A.; Umesono, K.; Mangelsdorf, D. J.; Evans, R. M.; *Nature*, **1992**, 355, 446-448.

[41] Yu, V. C.; Delsert, C.; Anderson, B.; Holloway, J. M.; Devary, O. V.; Naar, A. M.; Kim, S.Y.; Boutin, J. M.; Glass, C. K.; Rosenfeld M.G.; *Cell*, **1991**, 67, 1251-1266.

[42] Tsugawa, N.; Nakagawa, K.; Kawamoto, Y.; Tachibana, Y.; Hayashi, T.; Ozono, K.; Okano, T.; *Biol. Pharm. Bull.*, **1999**, 22, 371-377.

[43] Vargas, S.; Bouillon, R.; Van Baelen, H.; Raisz; L.G.; *Calcif. Tissue Int.*, **1990**, 47,164-168.

[44] Haddad, J. G.; Aden, D. P.; Aw, T. C.; *J. Bone Miner. Res.*, **1989**, 4, 243-247.

[45] Makishima, M.; Okabe-Kado, J.; Honma Y.; *Brit. J. Cancer*, **1998**, 77, 33-39.

[46] Okano, T.; Tsugawa, N.; Masuda, S.; Takeuchi, A.; Kobayashi, T.; Nishii, Y.; In

*Calcium Regulating Hormones. II. Calcium Transport, Bone Metabolism, and New Drugs. Contrib Nephrol.* Morii H, Ed.; Karger: Basel, **1991**, Vol. 91, pp. 116-122.

[47] Bouillon, R.; Allewaert, K.; Van Leeuwen, J.P.; Tan B.K.; Xiang, D.Z.; De Clereq, P.; Vandewalle, M.; Pols, H. A.; Bos, M. P.; Van Baelen, H.; *J. Biol. Chem.*, **1992**, 267, 3044-3051.

[48] Kobayashi, T.; Tsugawa, N.; Okano, T.; Masuda, S.; Takeuchi, A.; Kubodera, N.; Nishii, Y.; *J. Biochem.*, **1989**, 115, 373-380.

[49] Tachibana, Y.; Tsuji, M.; *Steroids*, **2001**, 66, 93-97.

[50] Bligh, E. G.; Dyer, W. J.; Lera, A.R.; *Can. J. Biochem. Physiol.*, **1959**, 37, 911-917.

[51] Reddy, G. S.; Tserng, K-Yi.; Thomas, B. R.; Dayal, R.; Norman, A.W.; *Biochemistry*, **1987**, 26, 324-331.

[52] Reddy G. S., Tserng K-Y., *Biochemistry*, **1986**, 25, 5328-5336.

[53] Onisco, B. L.; Esvelt, R. P.; Schnoes, H. K.; DeLuca, H. F.; *Biochemistry*, **1980**, 19, 4124-4130.

[54] Angeletti, R.; Bradshaw, R.A.; *Proc. Natl. Acad. Sci.*, **1971**, 68, 2417-2420.

[55] Brinton, R.D.; Yamazaki, R.S.; *Pharmacol. Res.*, **1998**, 15, 386-398.

[56] Lewin, G.R.; Barfdi, Y.A.; *Ann. Rev. Neuroscience*, **1996**, 19, 289-317.

[57] Tomoda, H.; Omura, S.; *Yakugaku Zashi*, **2000**, 120, 935-949.

[58] Koizumi, S.; Conttreras, M.L.; Matsuda, Y.; Hama, T.; Lazarovici, P.; Guroff, G.; *J. Neurosci.*, **1988**, 8, 715-721.

[59] Omura, S.; Sasaki, Y.; Iwai, Y.; Takeshima, H.; *J. Antibiotics*, **1995**, 48, 535-546.

[60] Kakeya, H.; Takahasi, I.; Okada, G.; Isono, K.; Osada, H.; *J. Antibiotics*, **1995**, 48, 733-735.

[61] Kawahara, F.; Saito, H.; Katsuki, H.; *Brain Research*, **1994**, 651, 101-107.

[62] Nes, W.D.; Norton, R.A.; Crumley, F.G.; Madigan, S.J.; Katz, P.R.; *Proc. Natl. Acad. Sci. USA.*, **1990**, 87, 7565-7569.

[63] Akihisa, T.; Matubara, Y.; Chosh, P.; Thakur, S.; Tamura, T.; Matsumoto, T.; *Steroids*, **1989**, 54, 625-638.

[64] Sucraw, W.; Slopianka, N.; Caldeira, P.P.; *Chem. Ber.*, **1975**, 108, 1101-1110.

[65] Tachibana, Y.; Kawahara, F.; *Symposium on the chemistry of natural products* (43rd) . **2001**, p503-508, (Osaka).

[66] Fryberg, M.; Oehlschager, A.C.; Unrau, A.M.; *J. Amer. Chem. Soc.*, **1973**, 95, 5747-5757.

[67] Choudhry, S.C.; Bolica, P.S.; Coffen, B.L.; Pocela, A.; Maehr, H.; Monchand, P.S.; Serico. L.; Yang, R.T.; J. *Org. Chem.*, **1993**, 58, 1496-1500.

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# CHARGED CAROTENOID SPECIES

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**ABSTRACT**: Carotenoids constitute a class of bioactive natural products. A short overview of naturally occurring carotenoids is presented. Although carotenoids are generally yellow-red isoprenoid polyene pigments, their light absorption range includes the UV-VIS-NIR region with charged carotenoids in the NIR region. The bioactivity of carotenoids are treated briefly as provitamins A and as proretinoids more generally, participation in photosynthesis and in photoprotection in a wider context, as well as in cell membrane stabilisation. Their actions as antioxidants and in health aspects receive much current interest. Some structure/activity relationships are considered. Carotenoid cation radicals are involved in photosynthesis and in antioxidant reactions. Charged carotenoids are treated in a systematic manner in this first comprehensive survey of the topic. Carotenoids with charged functional groups comprise naturally occurring carotenoid sulphates, which have been examined in detail, well known carotenoid carboxylic acids / carboxylates, synthetic phosphates, blue carotenoid oxonium ions studied in some detail, and blue carotenoproteins continuously investigated. Carotenoid cation radicals have received much interest in recent years. Theoretical calculations, their involvement in photosynthesis and *in vitro* preparation methods (electrochemically, *via* excited states, by Lewis acids or by oxidation with iodine) are referred to. Methods for characterisation include CV, NIR, EPR, ENDOR and resonance Raman spectroscopy. Properties and reactions of cation radicals are considered. For carotenoid dications are treated theoretical calculations, preparation by electrochemical oxidation or by treatment with $FeCl_3$ of $BF_3$ as suitable Lewis acids and their characterisation by NIR IR, and also recently by detailed NMR spectroscopy. Reactions of carotenoid dications with suitable nucleophiles are illustrated. Spinless carotenoid monocations are treated seperately. Included are theoretical calculations, hypothetical monocation intermediates in reactions with carotenols and 5,6-epoxides in acidic media, and characterised (NIR, NMR) monocations. Carotenoid anions including anion radicals and dianions are less studied. Theoretical calculations, preparation and spectral properties (NIR, Raman, EPR) are referred to. Relative to common carotenoids are carotenoids carrying a charge on the polyene chain particularly unstable and represent special challenges. This review gives 158 literature references, including 126 references since 1990.

# INTRODUCTION

## Scope

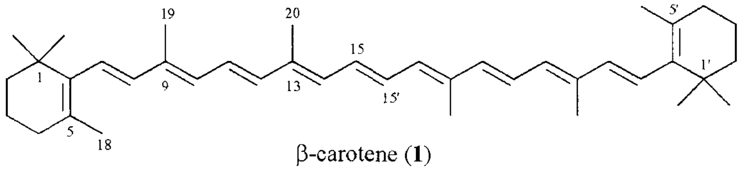
According to a recommended strategy for this series on studies in natural products chemistry, the present review is emphasising recent work and current projects involving charged carotenoid species in the authors' laboratory. However, the topic is treated in a broader context, referring to relevant studies by other groups.

Whereas carotenoids in general are often considered by non-specialists as unstable compounds, they may easily be studied when particular precautions are made [1]. This is also true for most subtypes with charged functional groups. The instability of carotenoid oxonium ions, radical cations, mono- and dications, however, represent special challenges. This review appears to be the first compilation on charged carotenoid species.

As this paper is prepared for a special volume on bioactive natural products, a brief overview on bioactivity of carotenoids is included, divided into known functions of carotenoids and to their actions on biological systems. Charged carotenoid species are involved in the function of carotenoids in photosynthesis and may turn out to be relevant unstable intermediates in other biological contexts, including antioxidant action.

## Naturally occurring carotenoids

Carotenoids are isoprenoid polyene pigments widely distributed in Nature. All carotenoids may formally be derived from β-carotene (**1**), Fig. 1, by simple chemical reactions.




β-carotene (**1**)

**Fig. (1).** β-Carotene (**1**) including the numbering of the carbon skeleton [2].

Most carotenoids possess $C_{40}$-skeletons, e.g. **2** [3]. Nor-carotenoids with atoms formally removed, e.g. **3** [4], apo-carotenoids with

abbreviated carbon skeleton e.g. **4** [5], diapo-carotenoids ($C_{30}$) and higher carotenoids with $C_{45}$ and $C_{50}$ skeletons e.g. **5** [6] are well known [7]. The examples in Fig. 2 are taken from own research.

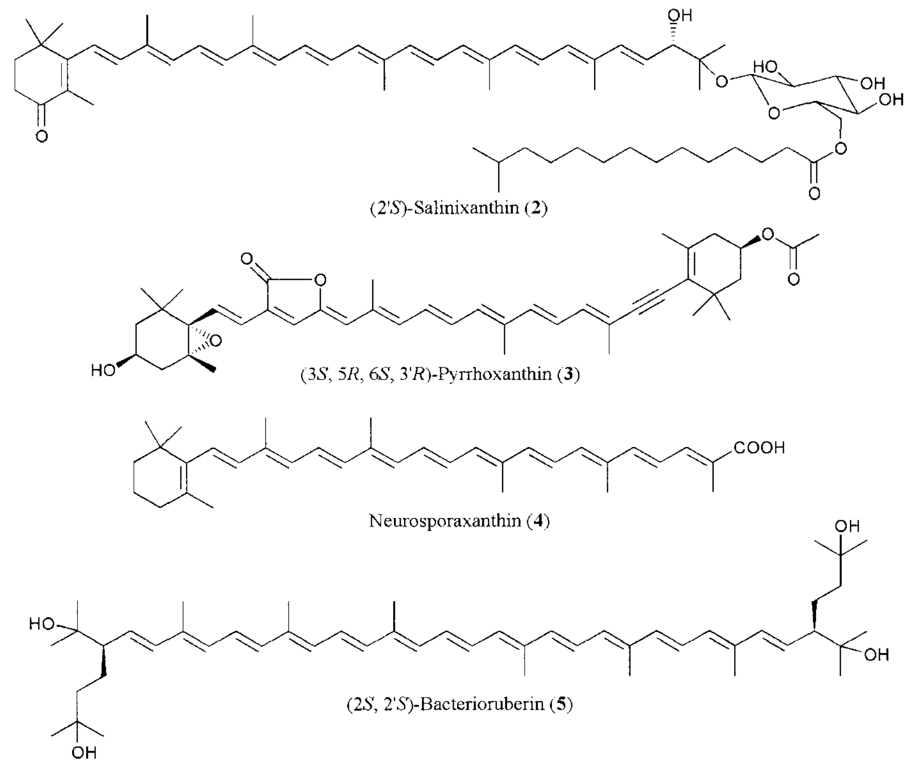



**Fig. (2).** Examples of naturally occurring carotenoids from different skeletal subclasses.

Functional groups include allene, acetylene, hydroxy, methoxy, epoxy, keto, aldehyde, carboxylate, lactone, acyl ester, glycoside, glycosyl ester and sulphate. Carotenoids with elements other than oxygen directly attached to the carbon skeleton have not been found in Nature.

The colour range of natural carotenoids goes from colourless - yellow - orange - red - blue, reflecting the chromophore involved. In Fig. 3 is illustrated major absorption regions of carotenoids in the UV-VIS-NIR regions.

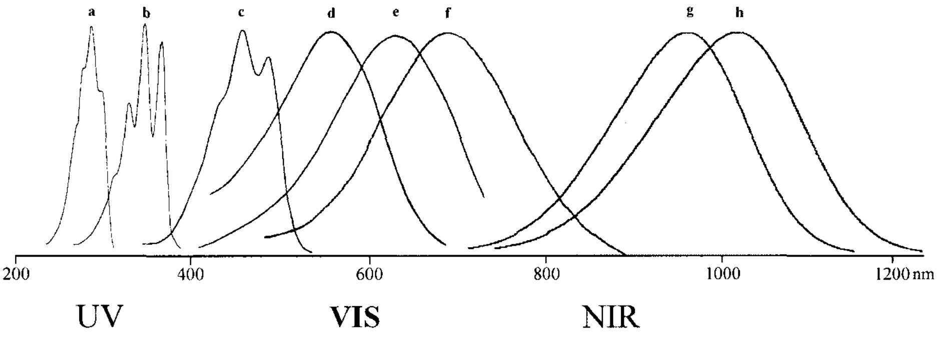



**Fig. (3).** The absorption of selected carotenoids in the range from 250 - 1200 nm.

The spectra presented in Fig. 3 are for **a** the aliphatic triene phytoene (**6**), **b** the aliphatic pentaene phytofluene (**7**), **c** β-carotene (**1**), all in hexane, **d** violerythrin (**8**, in acetone), **e** the astaxanthin based carotenoprotein crustacyanin [8,9] (**9**, in phosphate buffer), **f** fucoxanthin oxonium ion [10] (**10**, in methanol), **g** the dication [11] (**11**) and **h** the cation radical (**12**), both prepared from β-carotene (**1**). Structures are given in Fig. 4.

Proposed structures and exhaustive references to some 560 naturally occurring carotenoids studied until 1986, including trivial and semisystematic IUPAC names [2] have been compiled [7], as well as more recent additions [12]. A critical, selective treatment with key references will soon appear [13]. Extensive books on isolation and analysis including worked examples [1], applied spectroscopy [14], total synthesis [15] and biosynthesis and metabolism [16] have been published.

Carotenoids are synthesised *de novo* by all photosynthetic organisms including higher plants, phytoplankton and phototrophic bacteria as well as by certain other bacteria, yeasts and fungi. Carotenoids are selectively absorbed and metabolically modified in the food chain [17]. Animals cannot synthesise carotenoids *de novo*, but may contain specific carotenoids derived from dietary sources.

The distribution of carotenoids in plants [18] and animals [19] has been treated in monographs. The application of carotenoids in chemosystematic markers has received attention [18,20] and has been successful particularly for phototropic bacteria [21] and algae [18,22,23].

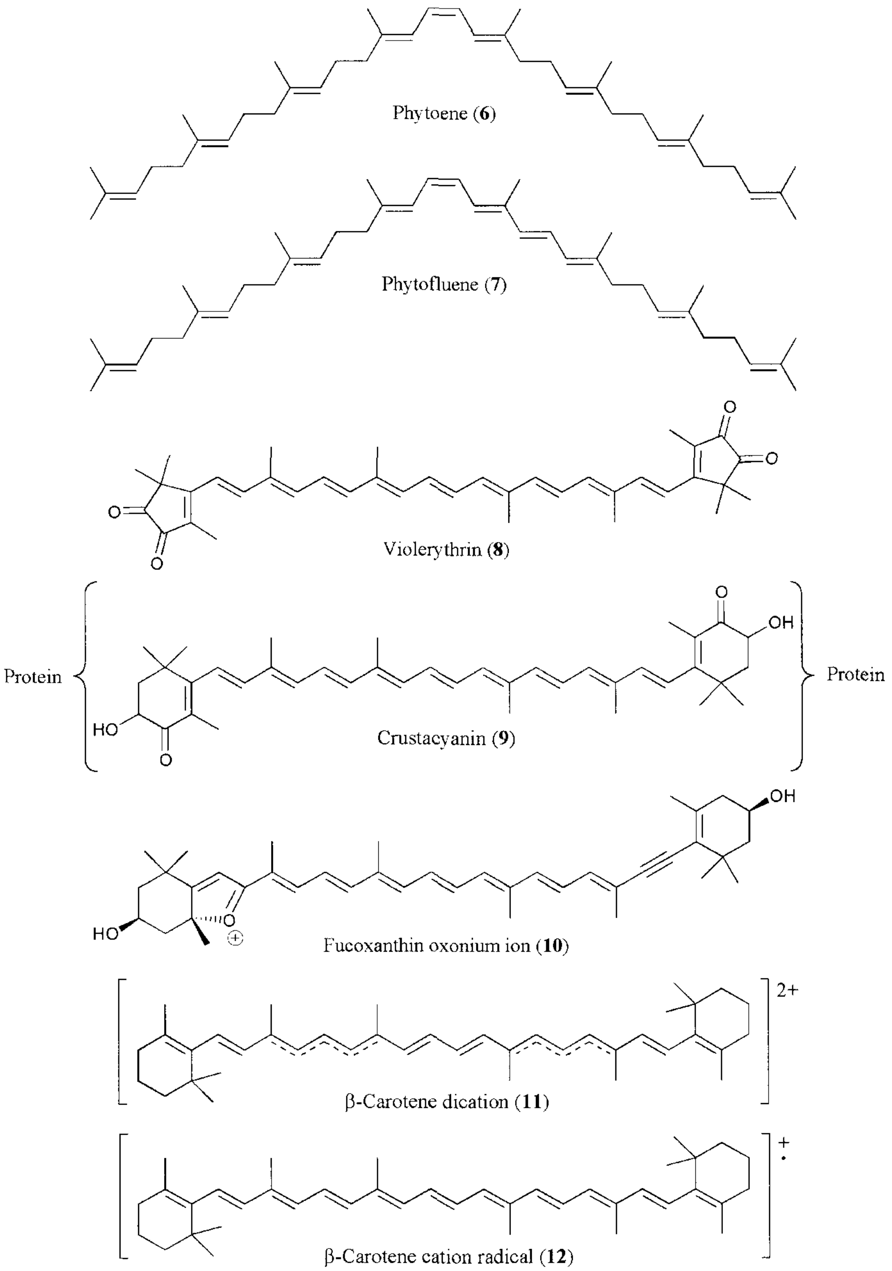



**Fig. (4).** Carotenoids referred to in Fig. 3 in order of increasing chromophore.

# BIOACTIVITY OF CAROTENOIDS

## Functions

### *Provitamins A*

Vitamin $A_1$ (retinol, **13**) is essential for the visual process and for normal growth and development of human beings. Carotenoids are the main dietary source of vitamin $A_1$. As provitamins $A_1$ serve particularly β-carotene (**1**) and also carotenoids with one molecular half of β-carotene (**1**) intact [24,25].

Freshwater fish further contain vitamin $A_2$ (3,4-didehydro-retinol, **14**). By means of isotopic labelling experiments and improved HPLC techniques it has recently been demonstrated that zeaxanthin (**15**), canthaxanthin (**16**) and astaxanthin (**17**) may be converted to vitamins $A_1$ and $A_2$ in vitamin A depleted rainbow trout [26]. Also in chicken may canthaxanthin (**16**) serve as a vitamin $A_1$ precursor [26].

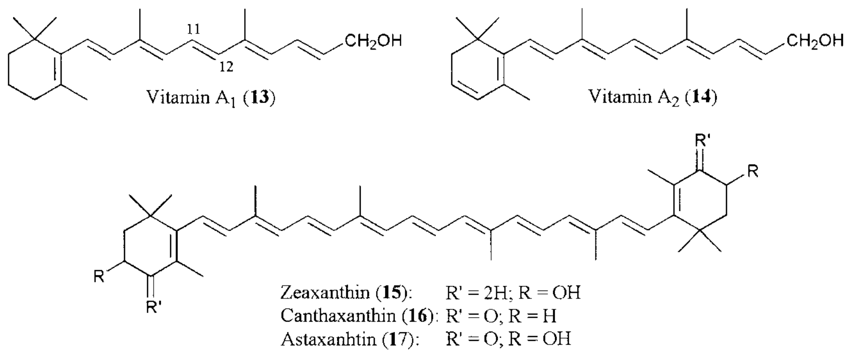


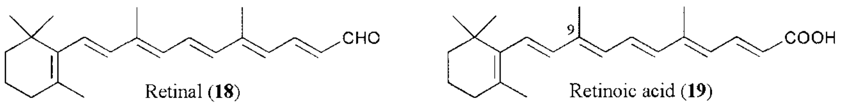

**Fig. (5).** Structures of Vitamin $A_1$ and $A_2$ and some special provitamins A in fish and chicken.

The metabolism of β-carotene (**1**) by central and by excentric cleavage to retinol (**13**) has been a topic of much debate [25]. Recently details of the central cleavage have been elegantly solved [27].

The role of retinal (**18**, vitamin $A_1$ aldehyde) in the visual process, involving *cis/trans* isomerisation of the sterically hindered C-11,12 double bond, is well established [28,29]. Besides the important function of retinal in visual signal transduction in animals is the function of energy production in halophilic bacteria, where the retinal-based bacterio-rhodopsin takes part in a light driven proton pump [30].

## *Proretinoids*

The function of certain carotenoids as provitamins A (retinol (**13**), 3,4-didehydroretinol (**14**) and retinal (**18**)), treated in the previous section, is during the last two decades further expanded as precursor role for additional biologically important retinoids, namely the all-*trans* and 9-*cis* isomers of retinoic acid (**19**), Fig. 6. Their function in growth regulation, development and cell differentiation *via* specific nuclear receptors are being clarified [24,31]. The origin of all-*trans* and 9-*cis* retinoic acid (**19**) has been considered [29,32,33].



Retinal (**18**) Retinoic acid (**19**)

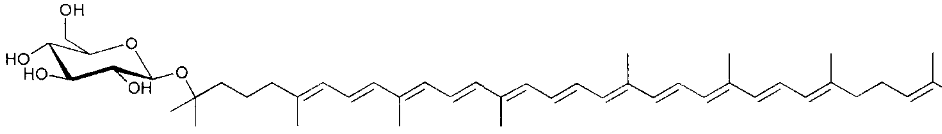
**Fig. (6).** Structure of retinal (11) and retinoic acid (12).

## *Photosynthesis*

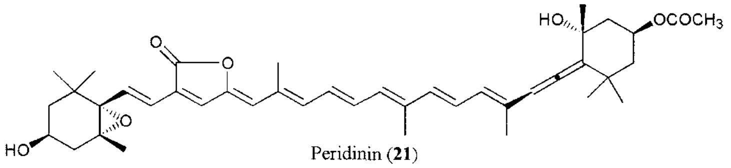
The state of the art is treated in a recent monograph [34]. Carotenoids serve two major functions in photosynthesis; light harvesting and photoprotection [34]. Recently the so-called xanthophyll cycle has been assigned a function in Photosystem 2 (PS2) [35]. PS2 is a membrane bound pigment-protein complex responsible for the oxidation of $H_2O$ to $O_2$ in oxygenic photosynthesis. The xanthophyll cycle involves a reversible 5,6-epoxidation of zeaxanthin (**15**) or its 7,8-didehydro acetylenic analogue. In light harvesting complexes the xanthophyll cycle serves to regulate light harvesting and subsequent energy dissipation in different light environments.

Specific carotenoids are involved in light harvesting in higher plants, algae and phototrophic bacteria. The detailed architecture of light harvesting complexes of e.g. rhodopin glucoside (**20**) containing phototropic bacteria and peridinin (**21**) containing dinoflagellates has lately been beautifully demonstrated by high resolution x-ray data [34].

Carotenoid cation radicals, which have lost one π-electron from the polyene chain, have been demonstrated in PS2 [36-39]. Moreover, the presence of carotenoid dications (lacking two π-electrons from the polyene chain) has been suggested in photosynthetic reaction centres [40].



Rhodopin glucoside (**20**)



Peridinin (**21**)

**Fig. (7).** Examples of carotenoids present in light harvesting complexes of phototropic bacteria and dinoflagellates.

## *Photoprotection*

It is well established that carotenoids serve as protectors against photodynamic destruction during photosynthesis and in certain non-phototrophic micro-organisms. The mechanism of photoprotection by carotenoids has been extensively treated [41-43]. The quenching of singlet oxygen $^1O_2$ by carotenoids play a prominent role. During photosynthesis, carotenoids prevent singlet oxygen from chlorophyll triplet states by rapid spin exchange and decay of the carotenoid triplet to the ground state.

In recent years the universal presence of various 15-*cis* carotenoids in the reaction centres of phototrophic organisms has been established and associated with photoprotective functions in PS2. It has been demonstrated that the 15-*cis* configuration has a unique property of efficient isomerisation to the all-*trans* configuration upon triplet excitation [43].

The structure/activity relationship for photoprotection is treated below.

## *Cell membrane stabilisation*

Long-chain $C_{50}$-carotenoids such as bacterioruberin (**5**) were first suggested as cell membrane stabilisers [44,45]. A model was subsequently proposed for cell membrane stabilisation by acylated carotenoid glucosides [46], such as salinixanthin (**2**) and thermozeaxanthin (**22**) [46], Fig. 8. The conjugated polyene chain was assumed to be located in the hydrophobic core of lipid bilayers with glucose moieties anchored in the hydrophilic region, whereas the branched fatty acid moieties were curved back into the hydrophobic region, thereby reinforcing the membrane [46].

Support has been obtained in experiments with natural thermozeaxanthin (**22**) containing mixed acyl residues [47] and with the pure *iso*-C15:0 acylated thermozeaxanthin (**23**), prepared by total synthesis, on a liposomal lipid bilayer [48].

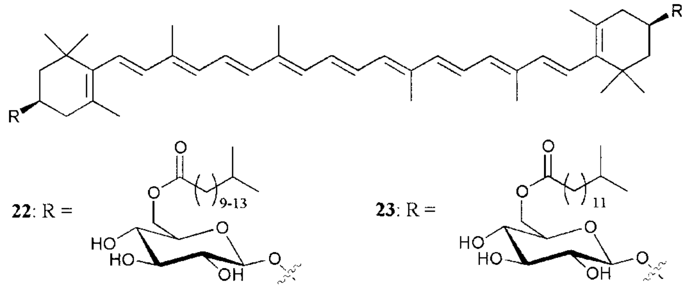



**Fig. (8).** Structures of thermozeaxanthins (**22** and **23**), serving cell wall stabilisation in thermophilic bacteria.

## *Miscellaneous*

Other functions of carotenoids are briefly mentioned here. These include phototropism and phototaxis, reproduction, camouflage colour, precursor role of neoxanthin (**38**, Fig. 16) for the plant growth hormone abscisic acid and the ant repellent grasshopper ketone [18,41], and cell-cell communication [49]. In recent years the function of zeaxanthin (**15**) and lutein (**25**) in the human retina has received much attention, cf. the section on Health aspects.

## Actions

### *Antioxidants*

Antioxidant and prooxidant properties of carotenoids have recently been reviewed [50,51]. Carotenoids serve as efficient quenchers of single oxygen. Their assumed function as antioxidants *in vivo* is largely based on model experiments.

Carotenoid radical cations are formulated as intermediates in various reactions between carotenoids and reactive oxygen species [50,52]. Carotenoids may interact with free radicals in three different ways [50]:

| | |
|---|---|
| Electron transfer: | $ROO^\bullet + Car \rightarrow ROO^- + Car^{+\bullet}$ |
| Hydrogen radical abstraction: | $ROO^\bullet + Car \rightarrow ROOH + Car^\bullet$ |
| Addition: | $ROO^\bullet + Car \rightarrow ROO\text{-}Car^\bullet$ |

In the human body are produced a range of reactive oxygen species, including $^1O_2$, $^\bullet OH$, $^\bullet O_2^-$, and $H_2O_2$ [53]. *In vitro* model studies aim at mimicking *in vivo* systems. The interaction of carotenoids with other dietary antioxidants have been studied, including vitamin C (ascorbic acid) and vitamin E (tocopherol, TOH) [54-56]. In the model proposed, the carotenoid (Car) is repairing vitamin E:

$$Car + TOH^{+\bullet} \rightarrow TOH + Car^{+\bullet}$$

For structure/activity relationships, see below.

### *Health aspects*

Considerable interest around carotenoids and health aspects originated in the finding that a diet based on vegetables and fruits is beneficial in health context [57]. So far, the carotenoids of main concern have been β-carotene (**1**), lycopene (**24**), lutein (**25**) and zeaxanthin (**15**), Fig. 9.

For β-carotene (**1**) supplementation studies, the findings from completed large scale trials indicate no overall benefits in well nourished human populations on the middle to late stages of carcinogenesis or on the incidence of cardiovascular disease [58]. The clinical use of β-carotene (**1**) as treatment for erythropoietic protoporphyria, in which $^1O_2$ is produced via sensitisation of free porphyrins in the skin, is well established [59].

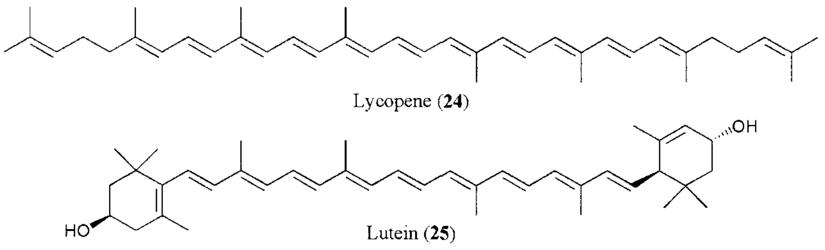



**Fig. (9).** Structures of some carotenoids of interest for health aspects.

Lycopene (**24**) is present in prostate tissue. During the last decade the possible role of lycopene (**24**) in prostate cancer prevention has received much interest [60-62].

The role of the macular carotenoids lutein (**25**) and zeaxanthin (**15**) in age-related macular degeneration has also been given much attention [63,64].

A recent volume on carotenoids in health and disease gives a broad treatment of topics related to this aspect [65].

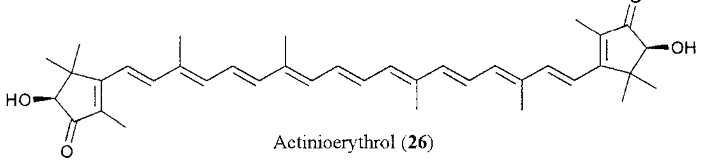
## Structure/activity relationships

### *Antioxidants*

Detailed *in vitro* studies [66] have demonstrated that a series of carotenoids fall into three groups on the basis of their antioxidant properties:

1. Little antioxidant properties:
   Colourless carotenes with short polyene chain, including phtyoene (**6**) and phytofluene (**7**).
2. Good antioxidant, but also prooxidant properties:
   β,β-carotene (**1**) > lycopene (**24**) > zeaxanthin (**15**).
3. Strong antioxidants with no prooxidant properties:
   canthaxanthin (**16**) ~ astaxanthin (**17**) > actinioerythrol (**26**).

The results have been rationalised [66].



Actinioerythrol (**26**)

**Fig. (10).** Structure of the good antioxidant actinoerythrol (**26**).

*Photoprotection*

Photoprotection by carotenoids is related to the length of the conjugated polyene chain. Experiments in this area have been thoroughly reviewed and the results tabulated [41]. For securing photoprotction a nonaene chromophore or a longer conjugated system is required, whereas carotenoids with shorter chromophores offer partial or no photo-protection.

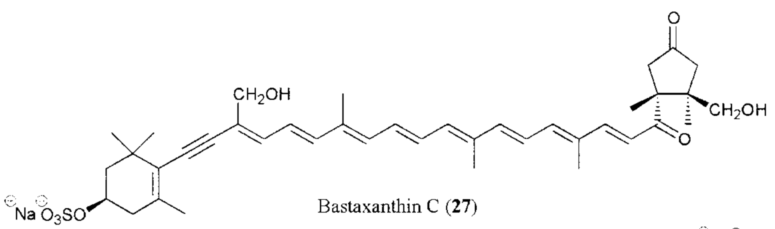
## CHARGED CAROTENOIDS

### Carotenoids with charged functional groups

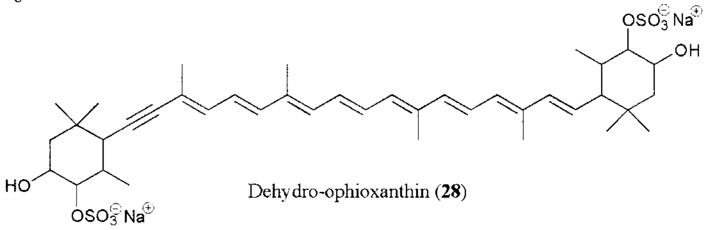
*Carotenoid sulphates*

Since the first reported naturally occurring carotenoid sulphate by our group in 1981 [67], several examples have been reported from marine sponges, echinoderms and bacteria [68]. The first structure established was for bastaxanthin C (**27**) [69]. The naturally occurring mono- and disulphates so far known contain either

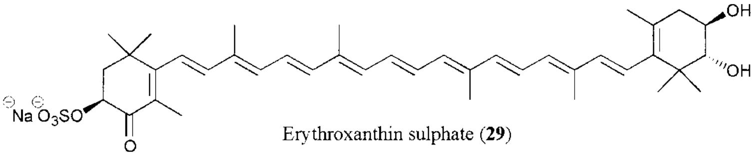
1. non-allylic secondary sulphate groups as in the bastaxanthins [68,69], or
2. as in dehydro-ophioxanthin (**28**) [70], or
3. a secondary sulphate group $\alpha$ to a carbonyl as in erythroxanthin sulphate (**29**) [71].




Bastaxanthin C (**27**)




Dehydro-ophioxanthin (**28**)




Erythroxanthin sulphate (**29**)

**Fig. (11).** Structure of some carotenoid sulphates.

The ionised sulphate group makes carotenoid sulphates strongly polar. This results in significant solubility in water with values up to 0.4 mg/ml. The high polarity and solubility properties determine the choice of isolation procedure. Special methods are employed and detailed isolation procedures available [68]. The isolation methods include column chromatography on Sephadex or silica, TLC and reversed phase HPLC. Sulphur analysis is also desirable. Further characterisation is carried out by spectroscopic methods.

The molecular ion can be detected by field desorption MS or by negative mode FAB. Upon EI ionisation no molecular ion is obtained, but distinct fragment ions are observed. A strong IR absorption at 1240 $cm^{-1}$ (S=O) is diagnostic. $^1H$ NMR spectra are only to a small extent influenced by the sulphate group.

The ionised carotenoid sulphate may be converted into the hydrogen sulphate by ion exchange. Hydrolysis of the sulphate may be accomplished enzymatically, or the sulphate group may alternatively be removed by acid hydrolysis [68].

Partial synthesis of carotenoid sulphates is effected from carotenols by reaction with a sulphur trioxide/pyridine complex prepared from chlorosulphinic acid and pyridine, followed by sodium salt formation by

addition of NaOH, or for alkali labile carotenoids by NaCl. The reaction is illustrated for zeaxanthin (**15**) in Fig. 12. Consistent with CD data does the carotenoid sulphate formation occur with retention of configuration [72].

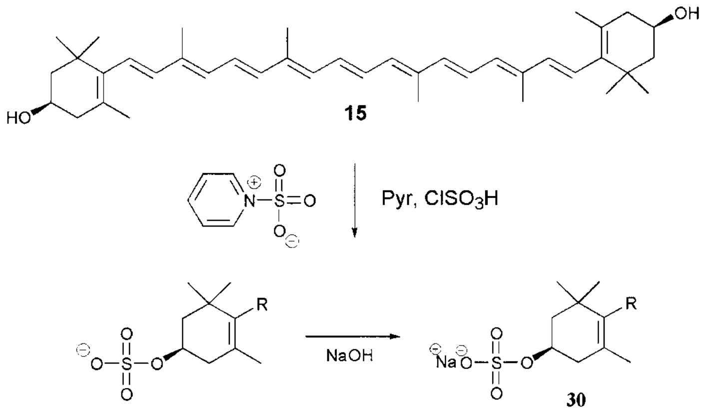



**Fig. (12).** Synthesis of zeaxanthin disulphate (**30**) from zeaxanthin (**15**).

Sulphate formation has been studied for carotenols with different end groups [73,74]. Carotenoids with non-allylic secondary hydroxy groups form stable sulphates. Less stable sulphates have been obtained from *tert*-carotenols, phenolic carotenols and non-allylic α-glycols. Unstable carotenoid sulphates are formed from *sec* or *tert* allylic carotenols. Solvolysis products of unstable carotenoid sulphates have been studied in some detail [73].

*Carotenoid carboxylic acids / carboxylates*

Amongst the apocarotenoids with less than 40 carbon atoms in the skeleton there are several examples of naturally occurring carotenoid mono- and dicarboxylic acids [7]. These include crocetin (**31**) from saffron, bixin (**32**) from *Bixa orellana*, azafrin (**33**) from roots of *Escobedria scabrifolia* and neurosporaxanthin (**4**) from yeasts (*Neurospora sp.*), Fig. 13.

Examples of carotenoid carboxylic acids are also found within the so-called diapocarotenoids ($C_{30}$) [7], exemplified by 4'-β-D-glucopyranosyl 4-hydrogen-7',8'-dihydro-4,4'-diapocarotene-4,4'-dioate (**34**) [5].

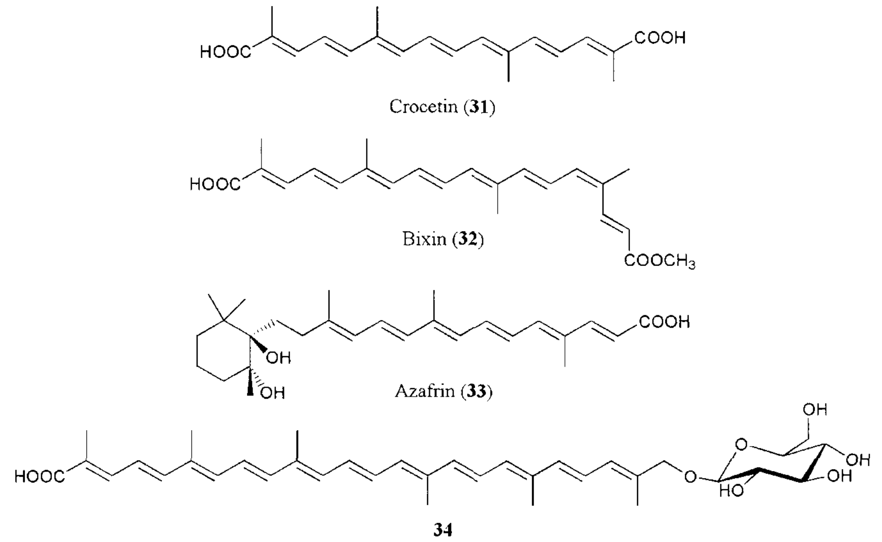



**Fig. (13).** Structures of selected carotenoid carboxylic acids.

As described for neurosporaxanthin (**4**) [75], exhibit carotenoid carboxylic acids acidic properties, and may therefore be converted to the methyl esters with diazomethane, Fig. 14. The polar carboxylate anion is obtained upon base treatment. Thus neurosporaxanthin (**4**) becomes completely hypophasic when distributed between petroleum ether and 1% NaOH in methanol-water (9:1) [75].

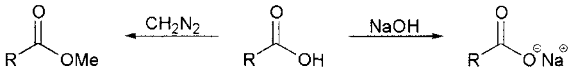



**Fig. (14).** Reactions of carotenoid carboxylic acids.

In the patent literature some interest concerns the application of salts of bixin (**32**) and crocetin (**31**).

*Carotenoid phosphates*

No carotenoid phosphates appear to have been encountered in Nature. The selenophosphate **35** was prepared synthetically from (3*R*,3'*R*)-zeaxanthin (**15**) in low yield in a Mitsunobu reaction with *O*,*O*-dipropyl phosphoroselenoic acid ester, Fig. 15. The Mitsunobu reaction occurs

with inversion of configuration, but elimination reactions were favoured [76].

The synthetic phospholipid (**36**) has been briefly reported [77].

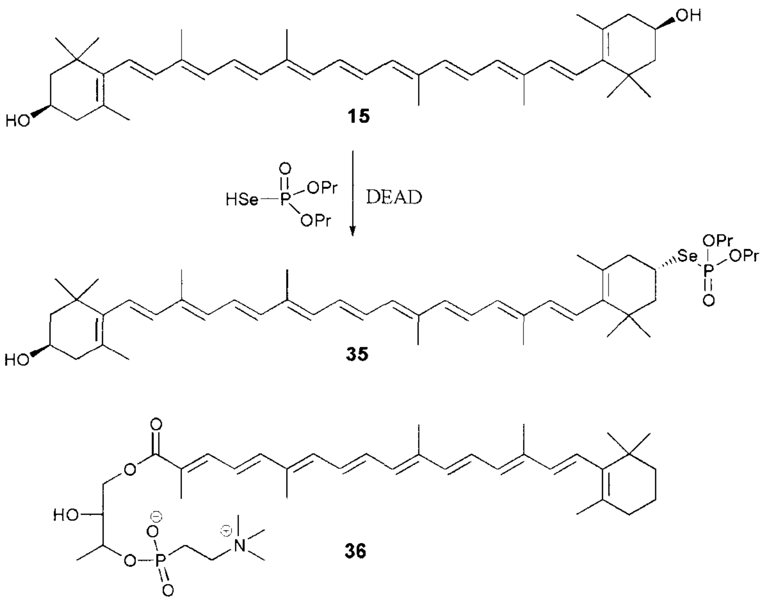



**Fig. (15).** Synthetic carotenoid phosphates.

## *Carotenoid oxonium ions*

A classical colour test for carotenoid 5,6-epoxides (e.g. violaxanthin (**37**)) is the formation of a dark blue colour upon treatment with strong acid [78,79]. The chemistry behind this reaction was elucidated in our laboratory, as illustrated for neoxanthin (**38**) in Fig. 16 [80]. Furanoxides such as neochrome (**39**) are intermediates in this reaction. The elimination of a hydride ion from C-8 of the furanoxide is a key step in the formation of the blue oxonium ion **40** and is effected by strong mineral acid (HCl), $CF_3COOH$ or by the hydride acceptor triphenylcarbenium tetrafluoroborate.

In $CF_3COOH$ the allenic end group of neoxanthin (**38**) is converted to the acetylenic end group [80] and with concentrated HCl, a chloride is added to the allene [10]. The blue oxonium ion **40** is converted to a yellow, cyclic hemiketal **41** by treatment with KOH. The hemiketal **41** is

very acid sensitive and readily converted to the blue oxonium ion **40**, Fig. 16.

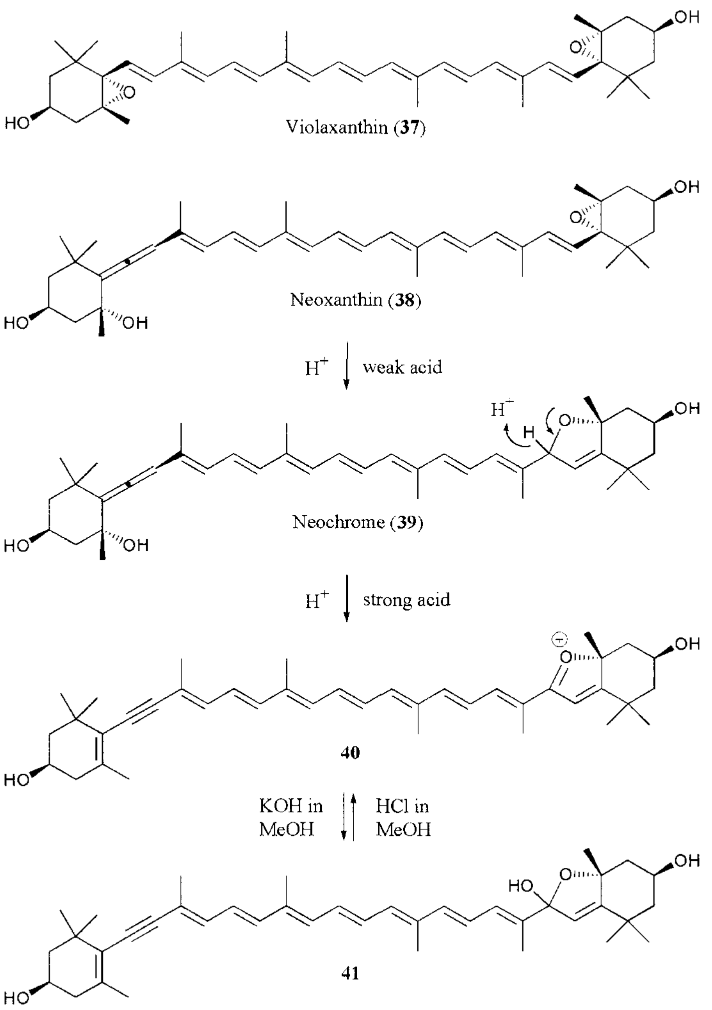



**Fig. (16).** Reaction mechanism for treatment of common carotenoid 5,6-epoxides (**37** and **38**) with acid [80].

The VIS spectrum of the yellow alkali treated product of the blue oxonium salt also contained products with octaene-, heptaene- and hexaene like chromophores [80], compatible with conjugate addition and charge delocalisation in the blue oxonium ion.

Fucoxanthin (**42**), lacking a double bond in the 7,8-position is an atypical 5,6-epoxide. However, the formation of blue oxonium ions with

strong acids has been studied and reaction mechanisms formulated *via* cyclic hemiketals [10], see Fig. 17.

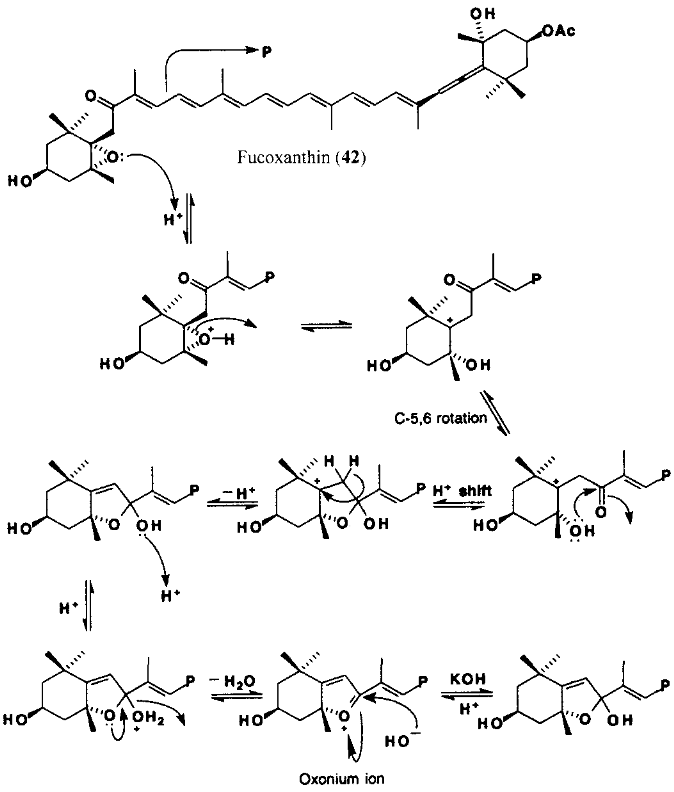



**Fig. (17).** Formation of blue oxonium ions via cyclic hemiketals upon treatment of fucoxanthin (**42**) with strong acid, and conversion of the blue oxonium ion to yellow hemiketal with base Reprinted with permission from [10]. Copyright 1994 Acta Chemica Scandinavica.

The striking colour change observed upon treatment of the yellow deacetylated fucoxanthin hemiketal (**43**) with acid, providing the blue oxonium ion (**10**, $\lambda_{max}$ 690 nm), followed by reversion to the yellow fucoxanthinol hemiketal (**43**) by treatment with base is illustrated in Fig. 18. The hemiketals exhibit characteristic fragmentation pattern by EIMS.

Well resolved $^1$H NMR spectra have not yet been achieved of the blue oxonium ions, and the structural proof relies on the chemical reactions and characterisation of the hemiketals.

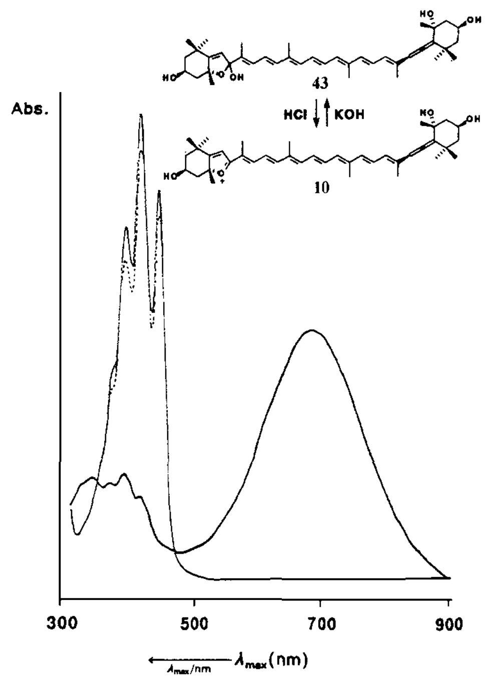



**Fig. (18).** Treatment of fucoxanthinol hemiketal (**43**, broken line) with acid, providing the blue oxonium ion (**10**, $\lambda_{max}$ 690 nm, solid line), followed by reversion to fucoxanthinol hemiketal (**43**) by treatment with base. Reprinted with permission from [10]. Copyright 1994 Acta Chemica Scandinavica.

## *Carotenoproteins*

Conjugated keto-carotenoids are complexed stoichiometrically with a simple protein in true carotenoproteins. Such carotenoproteins are encountered particularly in external tissues in the carapace of *Crustacea* and the skin of *Echinodermata.* The blue crustacyanin from lobster carapace is the best studied carotenoprotein, as described in several reviews [8,81,82]. Detailed isolations methods are given [83]. Our group has been involved in early studies on crustacyanin [84], asteriarubin [85], alloporin [86] and linckia-cyanin [87].

Astaxanthin (**17**) is not covalently bound in carotenoproteins and may be extracted from crustacyanin, providing a colourless apoprotein. The blue colour may be reconstituted with a range of conjugated ketocarotenoids [88]. All optical isomers (3*R*,3'*R*; 3*S*,3'*S* and the *meso*

form 3*R*,3'*S*) of astaxanthin (**17**) recombine equally well and result in identical CD spectra, determined by the chiral protein [84].

The basis of the colour shift from red (**17**: $\lambda_{max}$ 472 in hexane) to blue (crustacyanin $\lambda_{max}$ 632 in phosphate buffer) has received much attention. Recent results suggested a polarisation of the chromophore compatible with protonation of the keto groups [88]. These studies have been further refined by modern physical methods [9,89,90]. Supplementary X-ray data for the apoprotein [91] and for β-crustacyanin [92] have identified the contacts between astaxanthin (**17**) and the structural surroundings. The keto-oxygen atoms are within hydrogen bonding distance of two molecules of water and two histidine residues of the protein, acquiring a permanently induced dipole moment.

Structure **44**, Fig. 19, was recently proposed for protonated astaxanthin in crustacyanin with the delocalisation of the positive charge on the polyene chain mainly close to the rings [9]. Acidic amino acids were at that time suggested to be responsible for the protonation of the carotenoid.

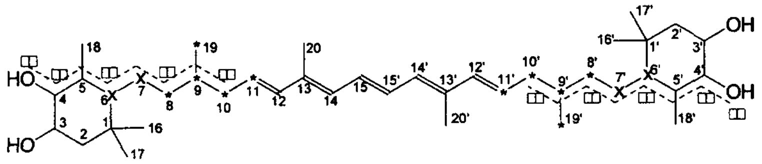


**Fig. (19).** Structure proposed for protonated astaxanthin in crustacyanin [9].

Consequently, the current picture concerning the bathochromic shift in carotenoproteins, such a crustacyanin (**9**), involves partial positive charge caused by hydrogen bonding of the astaxanthin (**17**) keto groups, cf. the blue oxonium ions discussed above.

## Carotenoid cation radicals

### *Theoretical calculations*

A carotenoid cation radical is in principle formed when one π-electron from the polyene chain is removed. *A priori* delocalisation of the unpaired electron would be predicted.

Austin Model 1 (AM1) geometry optimised coordinates were used as input to an INDO and an RHF-INDO/SP molecular orbital calculation for carotenoid cation radicals [93]. Lack of unpaired spin density in the cyclohexene rings was concluded. However, density functional (B3LYP) calculations for cation radicals of β-carotene (**1**) and canthaxanthin (**16**) with conjugated keto groups in the 4,4'-positions, support that the unpaired electron and the charge are delocalised over the whole polyene chain. The bond lengths were midway between the values for single and double bonds [94], see Fig. 29, including radical cations and dications of **1** and **16** (upper figures) and radical anions and dianions (lower figures).

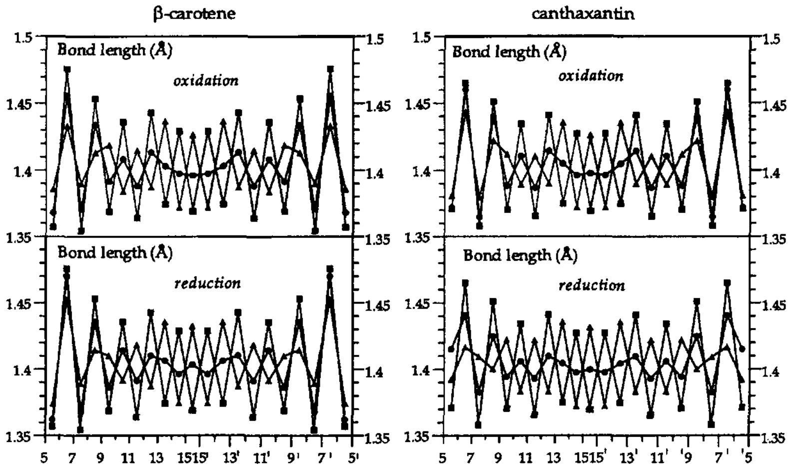



**Fig. (20).** Bond lengths in β-carotene and canthaxanthin and their ion radicals and di-ions, from gas-phase B3LYP calculations: Squares, neutral; circles, ion radical; triangles, di-ion. Reprinted with permission from [94]. Copyright 2001 American Chemical Society.

Also employing the hybrid density functional theory method (B3LYP) on the β-carotene radical cation, more refined results were obtained when taking rotation of the C6-C7-single bond into consideration [95], Fig. 21.

Structure **45** for the radical cation of **1** showed significant spin delocalisation to the β-ring end groups. The potential energy curve for rotation around the C6-C7 and C6'-C7' single bonds and the effect of this rotation on selected bond distances are reproduced in Fig. 21.

The current picture of the β-carotene radical cation is thus delocalisation of the unpaired spin delocalised over the entire conjugated

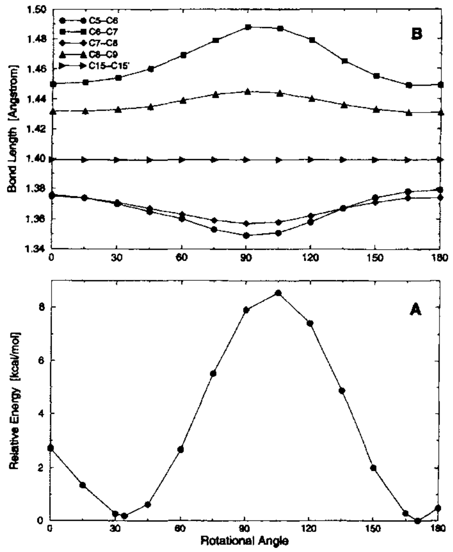
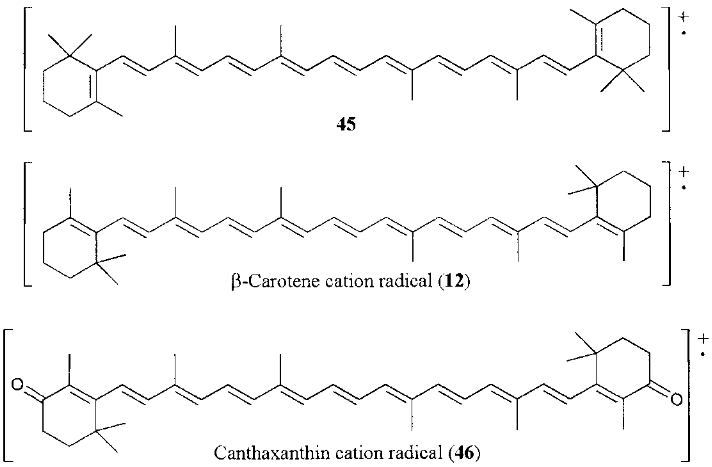



Fig. (21). Potential energy curve for rotation around the C6-C7 and C6'-C7' bonds (A), and the effect of this rotation on selected bond distances (B) of β-carotene cation radical. Reprinted with permission from [95]. Copyright 2001 American Chemical Society.

system based on the geometry of **12**. From density functional theory studies on canthaxanthin (**16**) and various apocarotenal derivatives it was concluded that the canthaxanthin radical cation had very similar properties to the unsubstituted β-carotene cation radical [96]. Structure **46** for the canthaxanthin radical cation may therefore be inferred, Fig. 22.



**45**

β-Carotene cation radical (**12**)

Canthaxanthin cation radical (**46**)

Fig. (22). Structures of carotenoid cation radicals.

*Cation radicals in Photosystem 2*

The presence of the β-carotene cation radical (**12**) in PS2 was mentioned in the section on photosynthesis. Since this discovery and work in the seventies, renewed interest has been received during the last decade.

Under particular conditions, β-carotene (**1**) serves as an alternative electron donor in the PS2 reaction centre, coping with highly oxidised intermediates formed during water oxidation. This redox role of β-carotene (**1**) in PS2 is unique amongst photosynthetic reaction centres. An overview on the electron transfer processes in PS2, emphasising those involving carotenoids, has recently been presented [97]. Methods employed for studies of the β-carotene cation radical (**12**) in PS2 include Resonance Raman [36,98,99], FT-IR [100], ENDOR [38] and EPR [37] spectroscopy, see under Methods below.

The properties recorded for β-carotene cation radical in PS2 compare well with those of β-carotene cation radical prepared by other methods.

*Preparation of carotenoid cation radicals*

A useful general guide for the generation of organic radical cations has been published [101]. Removal of one π-electron from a carotenoid has been achieved by various methods:

1. Electrochemical oxidation.

   The electrochemical properties of carotenoids has been reviewed [102,103]. Electrochemical data for several naturally occurring carotenoids including oxidation potentials, reaction rate constants and kinetic equilibrium constants were discussed. Conventional electrochemical techniques such as cyclic voltammetry (CV) were treated. The oxidation potential of neutral carotenoids, corresponding to the formation of cation radicals were 0.50 - 0.72 V *versus* SCE, and that of carotenoid cation radicals, corresponding to the formation of dications were 0.52 - 0.95 V *versus* SCE. Further details are available [104,105].

2. *Via* excited states.

   Cation radical formation of β-carotene (**1**) and other carotenoids were formed by photoexcitation at 308 nm in $CCl_4$ solution [106]. Cation radical formation of β-carotene (**1**) *via* the triplet state in chloroform

was studied [107]. Subsequently the involvement of the triplet state has been excluded [108].

3. By Lewis acids.

   Oxidation with more than two equivalents of ferric chloride is reported to result in the dication. The equilibration below was investigated for canthaxanthin (**16**) [109].

$$Car^{2+} + Car \leftrightarrows 2\ Car^{+\bullet}$$

   Radical cations of astaxanthin (**17**) were generated by oxidation with $FeCl_3$ at 150 K [110].

4. By oxidation with $I_2$.

   The result of this reaction has been debated. Due to the lack of EPR signal and C-I-stretch absorption in the IR spectrum, a cation $C_{40}H_{56}I^+$ without covalently bound iodine was considered. More recently a $C_{40}H_{56}I^+$ structure with iodine covalently bound or a dication $C_{40}H_{56}^{2+}$ were proposed [111-113] since *ab initio* calculations showed that addition of $I^+$ to a polyene is an endothermic process. Weak EPR signal for concentrated solutions at low temperature led to the proposed reaction [114]:

$$2\ C_{40}H_{56}\ (\mathbf{1}) + 3\ I_2 \rightarrow (2\ C_{40}H_{56}^{+\bullet} + 2I_3^-)$$

   The stoichiometry corresponds to a previous report for crystals of the complex from a benzene solution [115]. Cation radicals of β-carotene (**1**) and canthaxanthin (**16**) were again reported to be selectively formed in solutions by using iodine [109].

*Spectroscopic methods for characterisation*

For the characterisation of carotenoid cation radicals, there are four different spectroscopic techniques that have been most widely used;

1. Near infrared spectroscopy (NIR)
2. Electron paramagnetic resonance spectroscopy (EPR)
3. Electron nuclear double resonance spectroscopy (ENDOR)
4. Resonance Raman spectroscopy

The application of these techniques for studying carotenoid cation radicals are treated below.

A characteristic feature of carotenoid cation radicals is their electronic absorption in the NIR region, e.g. [36,40,55,103,116,117], in contrast to

the parent carotenoid, at times providing colourless solutions, cf. Fig. 3. NIR data available for β-carotene cation radical are compiled in Table 1.

**Table 1.** NIR data published for β-carotene cation radical (**12**).

| Solvent | $\lambda_{max}$ (nm) | ε | Ref |
|---|---|---|---|
| Hexane | 1040 | | [118] |
| $CHCl_3$ | 975 | | [119] |
| $CH_2Cl_2$ | 970,1425 | 63000, 2000 | [109] |
| DMSO | 942 | 16000 | [120] |
| Freon matrix | 813, 1280 | | [121] |

A hypsochromic shift with increasing solvent polarity is observed. Absorption curves for the cation radical and dication of β-carotene (**1**) are reported [109], see Fig. 23. The molar extinction coefficients for neutral β-carotene (**1**) and the charged species **11** and **12** are of comparable magnitude, Table 1, Fig. 23. INDO/S calculations for these spectra were performed and discussed.

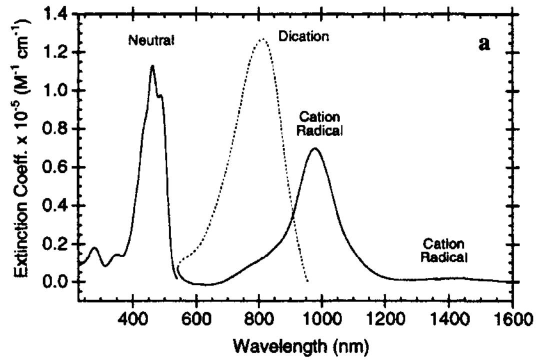



**Fig. (23).** Absorption spectra of β-carotene (**1**), its cation radical (**12**) and its dication (**11**) in dichloromethane. Reproduced with permission from [109]. Copyright 1996 American Chemical Society.

EPR is a general method for demonstrating the presence of free radicals with unpaired electrons [122]. EPR is widely used as a tool in studies involving carotenoid radicals [37,93,105,123-132].

The EPR spectrum of the β-carotene cation radical (**12**) imbedded in a mesoporous silica matrix was recently reported [123].

EPR data for the canthaxanthin cation radical (**46**), more recently generated in organic matrices, has been published [123-125]. A symmetrical unresolved EPR line at 9 GHz was assigned to a π-radical cation with electron density throughout the polyene chain [124].

EPR evidence was used to document photoinduced electron transfer between carotenoids (β-carotene (**1**) or canthaxanthin (**16**)) and solvent molecules [126].

ENDOR is a spectroscopic method used in studies of free radicals [122], and is used in modern studies on carotenoid cation radicals.

The ENDOR spectrum of the canthaxanthin cation radical (**46**) generated electrochemically in $CH_2Cl_2$ was first reported in 1993 [129]. The isotropic hyperfine coupling constants were in reasonable agreement with calculated values.

ENDOR spectra for positive ion radicals obtained by UV irradiation of β-carotene (**1**) and canthaxanthin (**16**) were discussed [125]. Improved techniques using continuous wave ENDOR (CW ENDOR) including 1D and 2D ESEEM techniques for cation radicals of canthaxanthin (**16**) and of an apocarotenal were recently reported [133].

The orientation of a carotenoid cation radical on solid support can be studied by ENDOR [123]. Pulsed ENDOR has been used for studies on carotenoid cation radicals in PS2 [38].

The principles, scope and limitations of Raman spectroscopy and its application in the carotenoid field has been discussed [134]. Time-resolved resonance Raman spectroscopy provides useful information on carotenoids in the excited state [135]. Differences between the resonance Raman spectra of a neutral carotenoid and its cation radical provide structural information on the cation radical.

A comprehensive resonance Raman study of carotenoid cation radicals generated from canthaxanthin (**16**) and various apocarotenoids is referred to [136]. The resonance Raman spectra of the carotenoid cation radicals were in general similar to the resonance Raman spectra of the excited triplet state of the carotenoid. Upon formation of the cation radical of canthaxanthin (**16**) and the apocarotenoids investigated, the C=C stretching vibrations were decreased by 30-40 $cm^{-1}$ whereas the C-C stretching vibrations were increased by 15-30 $cm^{-1}$ relative to the parent

carotenoids. The results were taken to support delocalisation of the unpaired electron over the polyene chain.

Resonance Raman was used in studies of the β-carotene cation radical in PS2 [36].

Time-resolved resonance Raman was used in an investigation of carotenoid cation radicals and triplet states [137].

*Properties and reactions of carotenoid cation radicals*

Spectroscopic and electrochemical properties of carotenoid cation radicals are already referred to.

The effects of the length of the polyene chain and acceptor substituents on the stability of carotenoid cation radicals have been studied [117]. For esters of conjugated carotenoid carboxylic acids, the stability of their cation radicals decreased with increasing length of the polyene chain. For conjugated aldehydes or nitriles there was no profound effect.

An intermediate role of cation radicals in *cis-trans* isomerisation of carotenoids has been considered [138]. AM1 molecular orbital calculations show that the energy barrier of *cis-trans* isomerisation are much lower in cation radicals (~20 kcal/mol) and dications (~0 kcal/mol) than in neutral carotenoids (~55 kcal/mol). HPLC analyses of the product mixture after bulk electrolysis of β-carotene (**1**), canthaxanthin (**16**) and apocarotenoids showed the presence of 15-*cis*, 13-*cis*, 9-*cis*, 9,13-di*cis* and all-*trans* isomers [138].

Reactions of carotenoid cation radicals were treated under anti-oxidants.

## Carotenoid dications

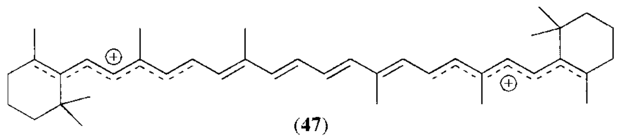
*Theoretical calculations*

A carotenoid dication is in principle formed when two π-electrons are removed from the polyene chain. In one electron processes a cation radical is an intermediate in this process. The dication is a spinless species.

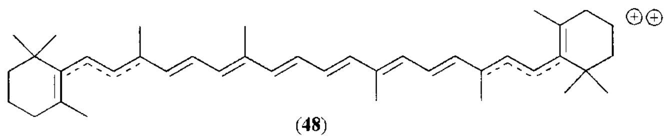
$$\text{Neutral carotenoid} \leftrightarrows \text{Cation radical} \leftrightarrows \text{Dication}$$

$$\text{Car} \leftrightarrows \text{Car}^{+\bullet} \leftrightarrows \text{Car}^{2+}$$

Structure **47** was suggested in 1992 for the β-carotene dication based on AM1 calculations [112,113]. The dication was depicted as a pair of charged solitons.



**(47)**



**(48)**

**Fig. (24).** Structures for β-carotene dication suggested on the basis of theoretical calculations.

Semi-empirical methods (AM1) were also employed for calculations of the doubly charged ion state [139] providing information on bond lengths, charge distribution and orbital energies, resulting in the suggestion of structure **48** for the dication of β-carotene (**1**). Doubly charged cations possessed two spinless charged symmetry equivalent areas, which developed more or less ideal bond equalisations. In the central part of the molecule, however, there was strong bond alternations, and in comparison with the neutral parent polyene a reversal of double and single bonds [139].

Both β-carotene (**1**) and **47** were depicted with rotated end groups [112,113], but rotation of the C-6,7 and C-6',7' single bonds upon formation of the dication of β-carotene (**1**) was not treated [112,113,139].

Calculated bond lengths for the dications of β-carotene (**1**) and canthaxanthin (**16**) are shown in Fig. 20 [94,140].

Application of multireference Møller-Plesset theory confirmed the general tendency observed in carotenoids that the first transition of the dications of polyenes is at a higher energy (shorter $\lambda_{max}$) than the intense transition of the radical cation and at a lower energy (longer $\lambda_{max}$) than the first allowed transition of the neutral species [141], cf. Fig. 23.

It should be mentioned that the structure and charge distribution of β-carotene (**1**) trication radical have been predicted by AM1 calculations and the optical spectrum estimated based on INDO/S calculations [140].

*Preparation of carotenoid dications*

Carotenoid dications have been prepared electrochemically [142,143], by $I_2$ doping [113] and by reactions with Lewis acids [11,109,141,144].

Electrochemically there is an equilibrium in dichloromethane solution between neutral carotenoid, cation radical and dication. The comproportionation constant ($k_{com}$) lies towards the formation of cation radicals for carotenoids possessing oxo groups or strong electron acceptor groups [142]. However, β-carotene (**1**) and carotenoids with electron donating groups exhibit $k_{com}$ in favour of the formation of dications [143].

$$Car^{++} + Car \overset{k_{com}}{\rightleftharpoons} 2\ Car^{+\bullet}$$

Ferric chloride ($FeCl_3$) is the Lewis acid most frequently employed [109,144]. Exclusive formations of dications were reported for carotenoids such as β-carotene (**1**) and canthaxanthin (**16**) when the carotenoids were oxidised with $FeCl_3$ [109]. The course of formation of canthaxanthin (**16**) dication and of astaxanthin (**17**) dication with $FeCl_3$ in hexafluoroisopropanol at low temperature was studied [144]. Recently, we have prepared β-carotene dication (**11**) by using $BF_3$-etherates in $CHCl_3$ at −20°C [11].

*Methods for characterisation*

Carotenoid dications may be characterised electrochemically, including cyclic voltammetry [40,94,105,139,140,145], by NIR spectroscopy [11, 40,109,112], by IR absorption [113], and recently by NMR spectroscopy [11].

NIR spectra are available for the dications of β-carotene (**1**) [11], canthaxanthin (**16**) [40,144], astaxanthin (**17**) [144], etc.

$I_2$ and $AsF_5$ doped β-carotene (**1**) films showed doping related IR absorption at 990, 1170 and 1470 $cm^{-1}$ [113].

Whereas free radicals like carotenoid cation radicals are not amenable to NMR analysis, was the first complete NMR analysis of β-carotene dication (**11**) recently accomplished by our group [11].

The detailed structure of the charge delocalised β-carotene dication (**11**) was established by COSY, HSQC, HMBC and 1D and 2D ROESY NMR techniques (600 MHz, $CDCl_3$, -20°C), leading to complete

assignments of $^1$H and $^{13}$C chemical shifts and $^3J_{H,H}$ coupling constants. Considerable downfield shifts were observed relative to β-carotene (**1**). The results were consistent with charge delocalisation in the C-5 – C-9 and C-5' – C-9' regions and with *retro* shifted double bonds in the central C-13 – C-13' region. The effect of the two delocalised charges on the chemical shifts (charge distribution) and bond distance ($^3J_{H,H}$) were discussed [11], see structure **11**, Fig. 4.

It is interesting to note that the dication structure **11** elucidated by NMR incorporates elements predicted by theoretical calculations including a pair of charged solitons (**47**, **48**) and reversal of double and single bonds in the central part of the molecule (**47**, **48**). Rotated C-6,7 and C-6',7' single bonds as calculated for the β-carotene cation radical (**12**), Fig. 21, was established for the dication **11**.

*Properties and reactions of carotenoid dications*

Spectroscopic properties are dealt with above.

The equilibrium between neutral carotenoid, cation radical and dication was already discussed [142,143]. More recently the effect of electrolytes and temperature on carotenoid dications were studied [40]. The stability of the β-carotene (**1**) dication at –25°C in $CHCl_3$ was remarkable, showing a decrease of less than 20% during 2h, as based on NIR spectra [11].

It was observed that electrochemical oxidation of all-*trans* β-carotene (**1**) and canthaxanthin (**16**) in $CH_2Cl_2$ leads to significant *trans-cis* isomerisation [105]. It was suggested that the isomerisation mechanism involved cation radicals and/or dications which could easily undergo geometrical isomerisation. This proposal was supported by AM1 molecular orbital calculations, which showed that the energy barrier from *trans* to *cis* is much lower in the cation radical and dication species than in the neutral carotenoid [105].

An alternative method to gain information on the structure of carotenoid dications is by studying their reactions with suitable nucleophiles [11]. We have recently investigated the products obtained when reacting the β-carotene dication (**11**) with $H_2O$ as a nucleophile in acetone providing isomerised isocryptoxanthin (**49**), mutatochrome (**50**) and strongly isomerised isocarotene (**51**), according to HPLC, Vis, MS and $^1$H NMR data. The formation of these products are rationalised in Fig.

25. The resulting *cis-trans* isomerisation is consistent with cationic intermediates.

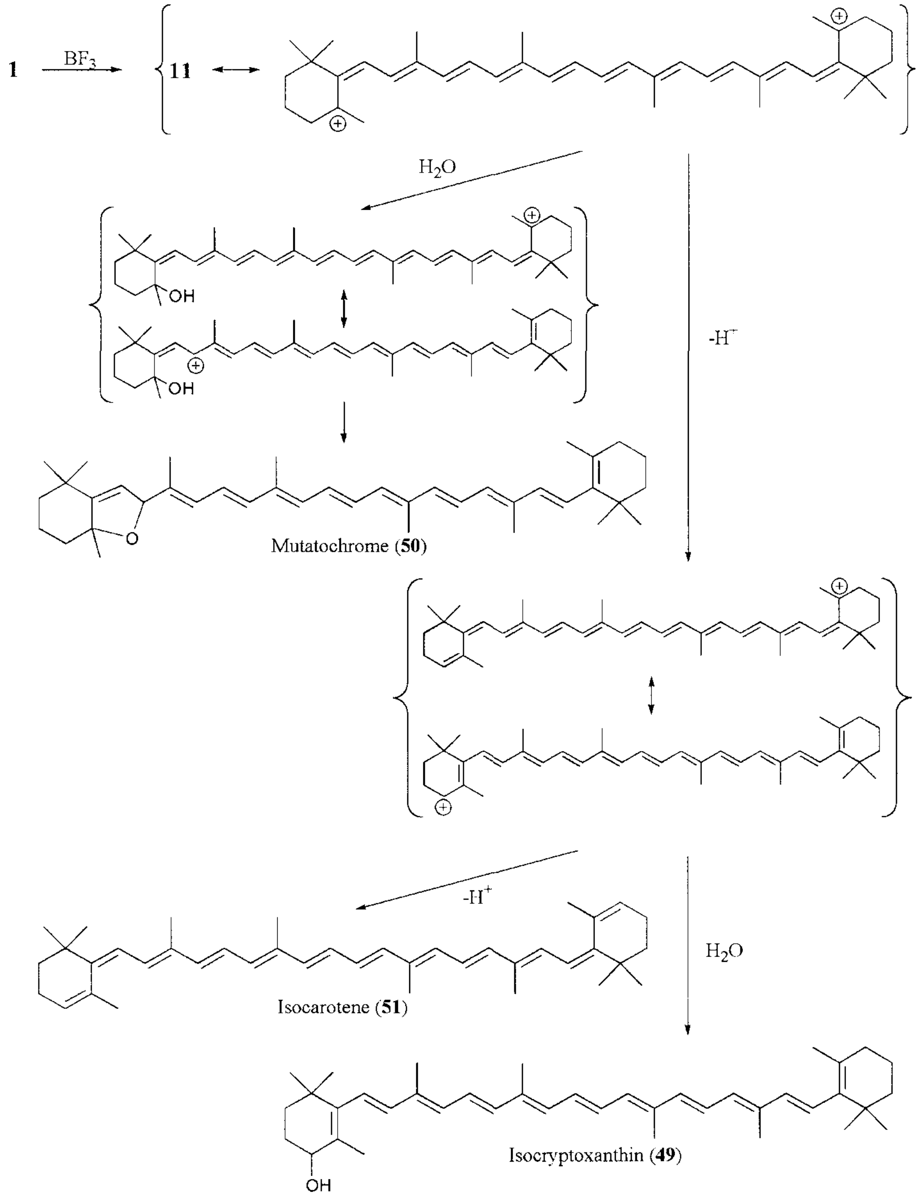



**Fig. (25).** Reactions of β-carotene dication (**11**) with water as nucleophile.

## Carotenoid monocations

### *Theoretical calculations*

AM1 calculated dipole moments, heats of formation, atomic charge and bond lengths of protonated carotenoids have been published for all protonation sites of β-carotene (**1**), canthaxanthin (**16**) and an apo-carotenal [116]. AM1 calculations predict that protonation can produce several protonated forms of each carotenoid. The protonation sites with the lowest enthalpy are C-7 < C-11 < C-5 < C-9 for **1** and C-7 < C-5 < C-11 < C-9 for **16** in the gas phase.

AM1 optimised carbocation geometry was used as input for single point INDO/S calculation of the carbocation optical spectra and the result discussed in light of experimental spectra. For protonation near the end of the polyene chain one single peak with longer $\lambda_{max}$ than for central protonation was predicted.

### *Hypothetical carotenoid monocations*

Carotenoid cation radicals are treated above. Considered here are spinless cations.

Allylic cations have been hypothetical intermediates in various common reactions with carotenoids, formed in acidic media in $S_N1$-type reactions. Two typical examples are mentioned here. Lutein (**25**) provides selectively the two C-3' methyl ethers (3'*R* and 3'*S*) upon treatment with acidic methanol [146]. The stereochemical result is compatible with an intermediate, planar, allylic cation, Fig. 26.

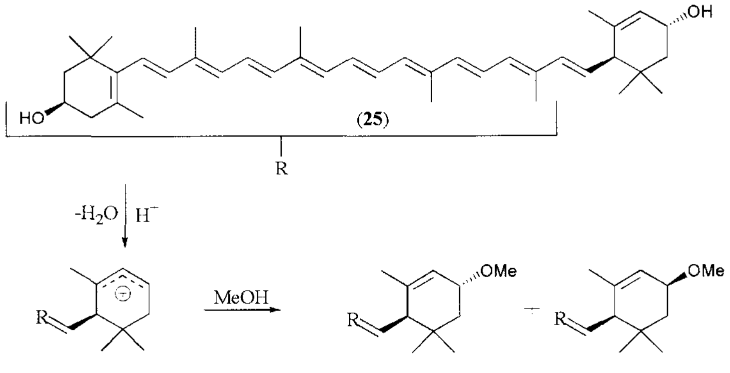
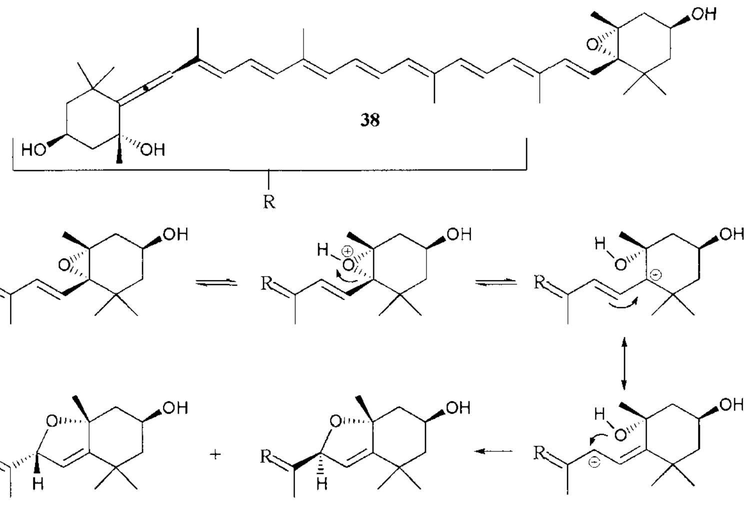



**Fig. (26).** Formation of lutein 3'-methyl ethers from lutein (**25**).

The selectivity of this reaction is rationalised by preferential formation of a delocalised allylic cation rather than a non-allylic *sec* C-3 cation.

The common 5,6-epoxide to 5,8-epoxide rearrangement [147] also provides two C-8 diastereomeric products in a 4:6 ratio. Thus neoxanthin (**38**) provides the 8'*S* and 8'*R* neochromes (**39**) upon treatment with weak acid, cf. Fig. 16, as predicted from an intermediary C-8' cation [148], Fig. 27.




(8'*R*)-**39** (8'*S*)-**39**

**Fig. (27).** Formation of neochromes (**39**) by treatment of neoxanthin (**38**) with weak acid.

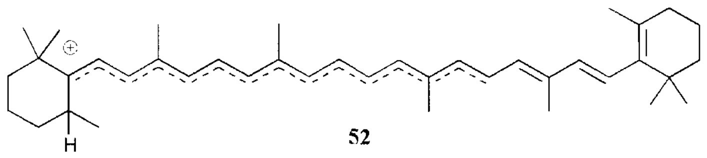
No such allylic carotenoid cations have been isolated and characterised.

*Characterised carotenoid monocations*

Experimental optical spectra in the NMR region are reported for protonated adducts of β-carotene (**1**), canthaxanthin (**16**) and an apocarotenal measured in benzene, $CH_2Cl_2$ and acetonitrile solutions at room temperature [116]. Extinction coefficients were estimated. For β-carotene (**1**) in the presence of 0.29 vol% trifluoroacetic acid at $\lambda_{max}$ 1022 nm in $CH_2Cl_2$, $\varepsilon = 1.86 \cdot 10^5$ was determined. The position of $\lambda_{max}$ for protonated **1** was in the same region as that of radical cations and was only slightly dependent on the solvent.

A series of aliphatic non-carotenoid polyenylic cations were prepared and studied by NMR [149]. The proton shifts were related to the excess positive charge density on the carbons to which the protons were bound. The value of the proportionality constant was found to be around 13-15 ppm per electron charge.

Recently, during studies on the reaction of β-carotene (**1**) with iodine, we succeeded in complete NMR characterisation of the monocation (**52**) [150], Chemical shifts and coupling constants were determined at –20°C in $CDCl_3$ by NMR (600 MHz) including COSY, HSQC, HMBC and ROESY techniques, leading to complete $^{1}H$ and $^{13}C$ assignments. Considerable downfield $^{1}H$ shifts of olefinic protons of **52** (6.4-8.1 ppm) relative to β-carotene (**1**) (6.1-6.7 ppm) are compatible with delocalised carbocations with maximum positive charge at C-6.




52

**Fig. (28).** β-Carotene monocation obtained by treatment of β-carotene (**1**) with iodine.

The mode of formation of this cation is not clear. Probably **52** was formed from the cation radical (**12**) by H• abstraction from an unidentified donor. Cation radicals have previously been claimed to be formed by oxidation of β-carotene (**1**) with iodine, cf. discussion under preparation of cation radicals.

Protonation of β-carotene (**1**) with trifluoroacetic acid in $CHCl_3$ provided a cation product with $\lambda_{max}$ 1050 nm and $^{1}H$ NMR data compatible with **52**, including the coupling pattern of the C-5 multiplet [150].

## Carotenoid anions

Carotenoid radical anions and dianions have been less extensively studied than the corresponding cations, and appear to have no biological relevance. A brief review on the topic is given below.

*Theoretical calculations*

In principle the uptake of one electron by a carotenoid molecule leads to an anion radical. Uptake of two electrons gives a dianion.

$$\text{Car} \xrightarrow{+e} \text{Car}^{\bullet -} \xrightarrow{+e} \text{Car}^{2-}$$

Gas phase B3LYP calculations on bond lengths of β-carotene (**1**) and canthaxanthin (**16**) and their anion radicals and dianions have been published [94], Fig. 20. It was concluded from UHF-MP2 *ab initio* calculations that the anion radical is symmetrical with the unpaired electron and charge delocalised mainly in the central part of the polyene chain, consistent with bond lengths between the values for single and double bonds. The dianions of **1** and **16** tended towards planarity relative to the non-planar geometry of the parent molecules, as judged by calculated C-5 - C-6 - C-7 - C-8 dihedral angles. The charge tended to be more concentrated in the dianion of canthaxanthin (**16**), while it remained more delocalised in the dianion of β-carotene (**1**).

NIR absorption spectra of radical anions of several carotenoids including phytoene (**6**), β-carotene (**1**), lycopene (**24**) and canthaxanthin (**16**) were calculated by Hückel and PPP methods and were in broad agreement with experimental findings [151].

*Preparation of carotenoid anions*

Radical anions of all-*trans* β-carotene (**1**) and its 15-*cis* isomer and of all-*trans* lycopene (**24**) were produced using pulse radiolysis techniques [118]. Pulse radiolysis was also employed for the preparation of radical anions of a series of carotenoids including phytoene (**6**) and canthaxanthin (**16**) [151]. More recently [152] the radical anion of β-carotene (**1**) was studied by pulse radiolysis, which appears to be the method of choice for the generation of carotenoid anion radicals.

An alternative preparation method is sodium doping employed for non-carotenoid polyenes, resulting in radical anions, **53**, as a charge transfer complex with one sodium ion [153], Fig. 29. It was shown that the charge storage depended upon the length of the polyene chain. Dimerisation of anion radicals was treated. Practical details concerning this method are available [154,155].

Dianions were also observed for all-*trans*-19,19',20,20'-tetranor-β-carotene (**54**), Fig. 29, lacking the four methyl substituents on the polyene chain of β-carotene (**1**) upon reduction with sodium in THF in a completely sealed glass cell [156].

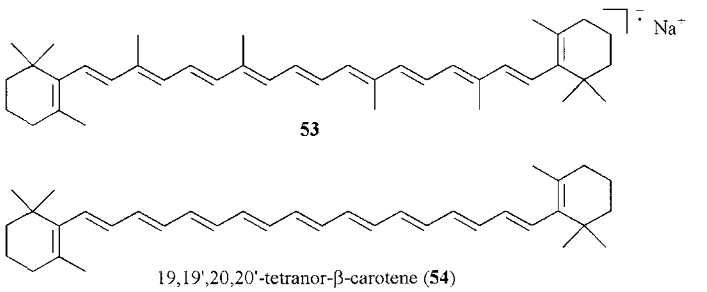



**Fig. (29).** Charge transfer complex of β-carotene anion radical with sodium ion (**53**) and structure of 19,19',20,20'-tetranor-β-carotene (**54**) used for the preparation of dianions.

## *Properties of carotenoid anions*

Already in 1975 were anion radicals shown to exhibit strong NIR absorption at ca. 150 nm shorter wavelengths than cation radicals [118]. The radical anions of β-carotene (**1**) and lycopene (**24**) exhibited $\lambda_{max}$ in hexane solution at 880 nm and at 950 nm respectively. Subsequent studies resulted in similar conclusions. The wavelength maxima of the absorption bands were linearly dependent on the number of conjugated double bonds [151]. Hypsochromic shifts were observed in methanol relative to hexane solutions [118,151].

The radical anion of 19,19',20,20'-tetranor-β-carotene (**54**) had an intense absorption at 1.46 eV and a weak absorption at 1.00 eV, while the dianion showed intense absorption at 1.59 eV [156]. This corresponds to 849 nm and 1240 nm, and 780 nm, respectively.

Raman spectra of the polyene **54** and its anion radical and dianion have been reported [156]. Inversion of bond alternation was suggested for the dianion. Absorption bands of the radical anion and dianion at 1275 $cm^{-1}$ was considered as a marker of negatively charged polyenes.

The radical anion of β-carotene (**1**) was detected by EPR. Spectral characteristics and decay constant were reported [157].

The application of NMR in carbanion chemistry has been reviewed [158]. No NMR data seems to have been reported for carotenoid anions.

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## REFERENCES

[1] Britton, G.; Liaaen-Jensen, S.; Pfander, H. Carotenoids Vol 1A: Isolation and analysis. Birkhäuser, Basel, **1995**.

[2] Weedon, B.C.L.; Moss, G.P. In *Carotenoids Vol 1A: Isolation and analysis*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1995**, pp. 27-70.

[3] Lutnaes, B.F.; Oren, A.; Liaaen-Jensen, S. *J. Nat. Prod.*, **2002**, *65*, 1340-1343.

[4] Englert, G.; Aakermann, T.; Liaaen-Jensen, S. *Magnet. Reson. Chem.*, **1993**, *31*, 910-915.

[5] Aasen, A.J.; Jensen, S.L. *Acta Chem. Scand.*, **1965**, *19*, 1843-1853.

[6] Johansen, J.E.; Liaaen-Jensen, S. *Tetrahedron Lett.*, **1976**, *12*, 955-958.

[7] Straub, O. In *Key to Carotenoids*; Pfander, H., Ed.; Birkhäuser: Basel, **1987**,

[8] Zagalsky, P.F.; Eliopoulos, E.E.; Findlay, J.B.C. *Comp. Biochem. Physiol. B*, **1990**, *97B*, 1-18.

[9] van Wijk, A.A.C.; Lugtenburg, J. In *Book of Abstracts, 13th Int. Carotenoid Symp.* Honolulu, **2002**, p. 16.

[10] Haugan, J.A.; Liaaen-Jensen, S. *Acta Chem. Scand.*, **1994**, *48*, 68-75.

[11] Lutnaes, B.F.; Bruas, L.; Krane, J.; Liaaen-Jensen, S. *Tetrahedron Lett.*, **2002**, *43*, 5149-5152.

[12] Kull, D.; Pfander, H. In *Carotenoids Vol 1A: Isolation and analysis*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1995**, Vol. *1A*, pp. 295-317.

[13] Britton, G.; Liaaen-Jensen, S.; Pfander, H. Carotenoid Handbook. Birkhäuser, Basel, **2003**.

[14] Britton, G.; Liaaen-Jensen, S.; Pfander, H. Carotenoids Vol 1B: Spectroscopy. Birkhäuser, Basel, **1995**.

[15] Britton, G.; Liaaen-Jensen, S.; Pfander, H. Carotenoids Vol 2: Synthesis. Birkhäuser, Basel, **1996**.

[16] Britton, G.; Liaaen-Jensen, S.; Pfander, H. Carotenoids Vol 3: Biosynthesis and metabolism. Birkhäuser, Basel, **1998**.

[17] Liaaen-Jensen, S. In *Carotenoids Vol 3: Biosynthesis and Metabolism*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1998**, pp. 359-371.

[18] Goodwin, T.W. The biochemistry of the carotenoids. Vol 1. Plants, 2nd ed. Chapman and Hall, London, **1980**.

[19] Goodwin, T.W. The biochemistry of the carotenoids Vol 2. Animals, 2nd ed. Chapman and Hall, London, **1984**.

[20] Liaaen-Jensen, S. In *Carotenoids Vol 3: Biosynthesis and Metabolism*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1998**, pp. 217-247.

[21] Schmidt, K. In *The photosynthetic bacteria*; Clayton, R.K.; Sistrom, W.R., Ed.; Plenum Press: New York, **1978**, pp. 729-750.

[22] Bjørnland, T.; Liaaen-Jensen, S. In *The chromophyte algae; Problems and Perspectives*; Green, J.C.; Leadbeater, B.S.C., Ed.; Clarendon Press: Oxford, **1989**, pp. 37-61.

[23] Liaaen-Jensen, S.; Egeland, E.S. In *Valuable chemicals from microalgae*; Cohen, Z., Ed.; Taylor & Francis: London, **1999**, pp. 145-172.

[24] Britton, G.; Liaaen-Jensen, S.; Pfander, H. In *Carotenoids Vol 3: Biosynthesis and metabolism*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1998**, pp. 1-12.

[25] Schweigert, F.J. In *Carotenoids Vol 3: Biosynthesis and metabolism*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1998**, pp. 249-284.

[26] Schiedt, K. In *Carotenoids Vol 3: Biosynthesis and metabolism*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1998**, pp. 285-358.

[27] Woggon, W.-D. *Pure Appl. Chem.*, **2002**, *74*, 1397-1408.

[28] Nakanishi, K. *Pure Appl. Chem.*, **1991**, *63*, 161-170.

[29] Rando, R.R. *Pure Appl. Chem.*, **1994**, *66*, 989-994.

[30] Mathies, R.A.; Lin, S.W.; Ames, J.B.; Pollard, W.T. *Annu. Rev. Biophys. Biophys. Chem.*, **1991**, *20*, 491-518.

[31] Redfern, C.P.F. In *Advances in organ biology*; Sherbet, G., Ed.; JAI Press: Connecticut, **1997**, Vol. *3*, pp. 35-77.

[32] Kliewer, S.A.; Umesono, K.; Evans, R.M.; Mangelsdorf, D.J. In *Vitamin A in health and disease*; Blomhoff, R., Ed.; Marcel Dekker: New York, **1994**, pp. 239-256.

[33] Drager, U.C.; Wagner, E.; McCaffery, P. *J. Nutr.*, **1998**, *128*, 463S-466S.

[34] Frank, H.A.; Young, A.J.; Britton, G.; Cogdell, R.J. The photochemistry of carotenoids. Kluwer, London, **1999**.

[35] Horton, P.; Ruban, A.V.; Young, A.J. In *The photochemistry of carotenoids*; Frank, H.A.; Young, A.J.; Britton, G.; Cogdell, R.J., Ed.; Kluwer: London, **1999**, pp. 271-291.

[36] Vrettos, J.S.; Stewart, D.H.; de Paula, J.C.; Brudvig, G.W. *J. Phys. Chem. B*, **1999**, *103*, 6403-6406.

[37] Lakshmi, K.V.; Reifler, M.J.; Brudvig, G.W.; Poluektov, O.G.; Wagner, A.M.; Thurnauer, M.C. *J. Phys. Chem. B*, **2000**, *104*, 10445-10448.

[38] Faller, P.; Maly, T.; Rutherford, A.W.; MacMillan, F. *Biochemistry*, **2001**, *40*, 320-326.

[39] Tracewell, C.A.; Cua, A.; Stewart, D.H.; Bocian, D.F.; Brudvig, G.W. *Biochemistry*, **2001**, *40*, 193-203.

[40] He, Z.; Kispert, L.D. *J. Phys. Chem. B*, **1999**, *103*, 10524-10531.

[41] Krinsky, N.I. In *Carotenoids*; Isler, O., Ed.; Birkhäuser: Basel, **1971**, pp. 669-716.

[42] Govindjee In *The photochemistry of carotenoids*; Frank, H.A.; Young, A.J.; Britton, G.; Cogdell, R.J., Ed.; Kluwer: London, **1999**, pp. 1-19.

[43] Koyama, Y.; Fujii, R. In *The photochemistry of carotenoids*; Frank, H.A.; Young, A.J.; Britton, G.; Cogdell, R.J., Ed.; Kluwer: London, **1999**, pp. 161-188.

[44] Ourisson, G.; Nakatani, Y. In *Carotenoids. Chemistry and biology*; Krinsky, N.I.; Adachi, K.; Sakai, M.; Shizuri, Y., Ed.; Plenum Press: New York, **1989**, pp. 237-245.

[45] Milon, A.; Wolff, G.; Ourisson, G.; Nakatani, Y. *Helv. Chim. Acta*, **1986**, *69*, 12-24.

[46] Yokoyama, A.; Sandmann, G.; Hoshino, T.; Adachi, K.; Sakai, M.; Shizuri, Y. *Tetrahedron Lett.*, **1995**, *36*, 4901-4904.

[47] Hara, M.; Yuan, H.; Yang, Q.; Hoshino, T.; Yokoyama, A.; Miyake, J. *Biochim. Biophys. Acta*, **1999**, *1461*, 147-154.

[48] Yamano, Y.; Sakai, Y.; Hara, M.; Ito, M. *J. Chem. Soc., Perkin Trans. 1*, **2002**, *17*, 2006-2013.

[49] Zhang, L.X.; Cooney, R.V.; Bertram, J.S. *Carcinogenesis*, **1991**, *12*, 2109-2114.

[50] Young, A.J.; Lowe, G.M. *Arch. Biochem. Biophys.*, **2001**, *385*, 20-27.

[51] Krinsky, N.I. *Nutrition*, **2001**, *17*, 815-817.

[52] Edge, R.; Truscott, T.G. In *The photochemistry of carotenoids*; Frank, H.A.; Young, A.J.; Britton, G.; Cogdell, R.J., Ed.; Kluwer: London, **1999**, pp. 223-234.

[53] Halliwell, B.; Gutteridge, J.M.C. Free radicals in biology and medicine. Oxford University Press, Oxford, **1999**.

[54] Bohm, F.; Edge, R.; McGarvey, D.J.; Truscott, T.G. *FEBS Lett.*, **1998**, *436*, 387-389.

[55] Edge, R.; Land, E.J.; McGarvey, D.; Mulroy, L.; Truscott, T.G. *J. Am. Chem. Soc.*, **1998**, *120*, 4087-4090.

[56] Burke, M.; Edge, R.; Land, E.J.; Truscott, T.G. *J. Photochem. Photobiol. B*, **2001**, *60*, 1-6.

[57] Nishino, H.; Tokuda, H.; Satomi, Y.; Masuda, M.; Bu, P.; Onozuka, M.; Yamaguchi, S.; Okuda, Y.; Takayasu, J.; Tsuruta, J.; Okuda, M.; Ichiishi, E.; Murakoshi, M.; Kato, T.; Misawa, N.; Narisawa, T.; Takasuka, N.; Yano, M. *Pure Appl. Chem.*, **1999**, *71*, 2273-2278.

[58] Hennekens, C.H. *Pure Appl. Chem.*, **1997**, *69*, 2141-2144.

[59] Krinsky, N.I. *Free Radic. Biol. Med.*, **1989**, *7*, 617-635.

[60] Miller, E.C.; Hadley, C.W.; Schwartz, S.J.; Erdman, J.W.J.; Boileau, T.W.-M.; Clinton, S.K. *Pure Appl. Chem.*, **2002**, *74*, 1435-1441.

[61] Giovannucci, E. *Pure Appl. Chem.*, **2002**, *74*, 1427-1434.

[62] Kucuk, O.; Sarkar, F.H.; Sakr, W.; Khachik, F.; Djuric, Z.; Banerjee, M.; Pollak, M.N.; Bertram, J.S.; Wood, D.P.J. *Pure Appl. Chem.*, **2002**, *74*, 1443-1450.

[63] Landrum, J.T.; Bone, R.A.; Chen, Y.; Herrero, C.; Llerena, C.M.; Twarowska, E. *Pure Appl. Chem.*, **1999**, *71*, 2237-2244.

[64] Bernstein, P.S. *Pure Appl. Chem.*, **2002**, *74*, 1419-1425.

[65] Krinsky, N.I.; Mayne, S.T.; Sies, H. Carotenoids in health and disease. Marcel Dekker, New York, **2003**.

[66] Martin, H.D.; Ruck, C.; Schmidt, M.; Sell, S.; Beutner, S.; Mayer, B.; Walsh, R. *Pure Appl. Chem.*, **1999**, *71*, 2253-2262.
[67] Ramdahl, T.; Kazlauskas, R.; Bergquist, P.; Liaaen-Jensen, S. *Biochem. Syst. Ecol.*, **1981**, *9*, 211-213.
[68] Liaaen-Jensen, S.; Hertzberg, S. In *Carotenoids Vol 1A: Isolation and analysis*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1995**, pp. 283-286.
[69] Hertzberg, S.; Ramdahl, T.; Johansen, J.E.; Liaaen-Jensen, S. *Acta Chem. Scand. B.*, **1983**, *B37*, 267-279.
[70] D'Auria, M.V.; Minale, L.; Riccio, R.; Uriarte, E. *J. Nat. Prod.*, **1991**, *54*, 606-608.
[71] Takaichi, S.; Furihata, K.; Ishidsu, J.; Shimada, K. *Phytochemistry*, **1991**, *30*, 3411-3415.
[72] Ramdahl, T.; Liaaen-Jensen, S. *Acta Chem. Scand. B.*, **1980**, *B 34*, 773-774.
[73] Hertzberg, S.; Berger, H.; Liaaen-Jensen, S. *Acta Chem. Scand. B.*, **1985**, *B39*, 725-734.
[74] Liaaen-Jensen, S. In *Carotenoids Vol 2: Synthesis*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1996**, pp. 295-300.
[75] Taylor, R.F.; Davies, B.H. *Can. J. Biochem.*, **1982**, *60*, 675-683.
[76] Sliwka, H.-R. *Acta Chem. Scand.*, **1997**, *51*, 345-347.
[77] Foss, B.J.; Partali, V.; Sliwka, H.-R.; Martin, H.-D.; Mayer, B. In *Book of Abstracts, 13th Int. Carotenoid Symp.* Honolulu, **2002**, p. 39.
[78] Karrer, K.; Jucker, E. Carotenoids. Elsevier, New York, **1950**.
[79] Strain, H.H. *Arch. Biochem. Biophys.*, **1954**, *48*, 458-468.
[80] Haugan, J.A.; Liaaen-Jensen, S. *Acta Chem. Scand.*, **1994**, *48*, 152-159.
[81] Cheesman, D.F.; Lee, W.L.; Zagalsky, P.F. *Biol. Rev. Camb. Philos. Soc.*, **1967**, *42*, 131-160.
[82] Britton, G.; Armitt, G.M.; Lau, S.Y.M.; Patel, A.K.; Shone, C.C. In *Carotenoid chemistry and biochemistry*; Britton, G.; Goodwin, T.W., Ed.; Pergamon Press: Oxford, **1982**, pp. 237-251.
[83] Zagalsky, P.F. In *Carotenoids Vol 1A: Isolation and analysis*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1995**, pp. 287-294.
[84] Berger, H.; Roenneberg, H.; Borch, G.; Liaaen-Jensen, S. *Comp. Biochem. Physiol.*, **1982**, *71B*, 253-258.
[85] Renstroem, B.; Tauber, J.D.; Elgsaeter, A.; Liaaen-Jensen, S. *Biochem. Syst. Ecol.*, **1979**, *7*, 147-148.
[86] Ronneberg, H.; Borch, G.; Fox, D.L.; Liaaen-Jensen, S. *Comp. Biochem. Physiol. B*, **1979**, *62B*, 309-312.
[87] Zagalsky, P.F.; Haxo, F.; Hertzberg, S.; Liaaen-Jensen, S. *Comp. Biochem. Physiol. B*, **1989**, *93B*, 339-353.
[88] Britton, G.; Weesie, R.J.; Askin, D.; Warburton, J.D.; Gallardo-Guerrero, L.; Jansen, F.J.; de Groot, H.J.M.; Lugtenburg, J.; Cornard, J.-P.; Merlin, J.-C. *Pure Appl. Chem.*, **1997**, *69*, 2075-2084.
[89] Weesie, R.J.; Verel, R.; Jansen, F.J.H.M.; Britton, G.; Lugtenburg, J.; de Groot, H.J.M. *Pure Appl. Chem.*, **1997**, *69*, 2085-2090.

[90] Weesie, R.J.; Merlin, J.C.; De Groot, H.J.M.; Britton, G.; Lugtenburg, J.; Jansen, F.J.H.M.; Cornard, J.P. *Biospectrosc.*, **1999**, *5*, 358-370.
[91] Cianci, M.; Rizkallah, P.J.; Olczak, A.; Raftery, J.; Chayen, N.E.; Zagalsky, P.F.; Helliwell, J.R. *Acta Crystallogr. D Biol. Crystallogr.*, **2001**, *D57*, 1219-1229.
[92] Cianci, M.; Rizkallah, P.J.; Olczak, A.; Raftery, J.; Chayen, N.E.; Zagalsky, P.F.; Helliwell, J.R. *Proc. Natl. Acad. Sci. U. S. A.*, **2002**, *99*, 9795-9800.
[93] Piekara-Sady, L.; Khaled, M.M.; Bradford, E.; Kispert, L.D.; Plato, M. *Chem. Phys. Lett.*, **1991**, *186*, 143-148.
[94] Hapiot, P.; Kispert, L.D.; Konovalov, V.V.; Saveant, J.-M. *J. Am. Chem. Soc.*, **2001**, *123*, 6669-6677.
[95] Himo, F. *J. Phys. Chem. A*, **2001**, *105*, 7933-7937.
[96] Guo, J.-D.; Luo, Y.; Himo, F. *Chem. Phys. Lett.*, **2002**, *366*, 73-81.
[97] Tracewell, C.A.; Vrettos, J.S.; Bautista, J.A.; Frank, H.A.; Brudvig, G.W. *Arch. Biochem. Biophys.*, **2001**, *385*, 61-69.
[98] Pascal, A.; Telfer, A.; Barber, J.; Robert, B. *FEBS Lett.*, **1999**, *453*, 11-14.
[99] Telfer, A.; Frolov, D.; Barber, J.; Robert, B.; Pascal, A. *Biochemistry*, **2003**, *42*, 1008-1015.
[100] Noguchi, T.; Mitsuka, T.; Inoue, Y. *FEBS Lett.*, **1994**, *356*, 179-182.
[101] Davies, A.G. *J. Chem. Res.*, **2001**, *7*, 253-261.
[102] Liu, D.; Kispert, L.D. *Recent Res. Dev. Electrochem.*, **1999**, *2*, 139-157.
[103] Liu, D.; Gao, Y.; Kispert, L.D. *J. Electroanal. Chem.*, **2000**, *488*, 140-150.
[104] Grant, J.L.; Kramer, V.J.; Ding, R.; Kispert, L.D. *J. Am. Chem. Soc.*, **1988**, *110*, 2151-2157.
[105] Gao, G.; Wei, C.C.; Jeevarajan, A.S.; Kispert, L.D. *J. Phys. Chem.*, **1996**, *100*, 5362-5366.
[106] Jeevarajan, A.S.; Kispert, L.D.; Avdievich, N.I.; Forbes, M.D.E. *J. Phys. Chem.*, **1996**, *100*, 669-671.
[107] Fujii, R.; Koyama, Y.; Mortensen, A.; Skibsted, L.H. *Chem. Phys. Lett.*, **2000**, *326*, 33-38.
[108] Gurzadyan, G.G.; Steenken, S. *Phys. Chem. Chem. Phys.*, **2002**, *4*, 2983-2988.
[109] Jeevarajan, J.A.; Wei, C.C.; Jeevarajan, A.S.; Kispert, L.D. *J. Phys. Chem.*, **1996**, *100*, 5637-5641.
[110] Krawczyk, S. *Chem. Phys. Lett.*, **1998**, *294*, 351-356.
[111] Ehrenfreund, E.; Hagler, T.W.; Moses, D.; Wudl, F.; Heeger, A.J. *Synth. Met.*, **1992**, *49*, 77-82.
[112] Ehrenfreund, E.; Moses, D.; Heeger, A.J.; Cornil, J.; Bredas, J.L. *Chem. Phys. Lett.*, **1992**, *196*, 84-90.
[113] Ehrenfreund, E.; Moses, D.; Lee, K.; Heeger, A.J.; Cornil, J.; Bredas, J.L. *Synth. Met.*, **1993**, *57*, 4707-4713.
[114] Ding, R.; Grant, J.L.; Metzger, R.M.; Kispert, L.D. *J. Phys. Chem.*, **1988**, *92*, 4600-4606.
[115] Matsuyama, T.; Sakai, H.; Yamaoka, H.; Maeda, Y. *J. Chem. Soc. [Dalton Trans.]*, **1982**, *1*, 229-231.
[116] Konovalov, V.V.; Kispert, L.D. *J. Chem. Soc., Perkin Trans. 2*, **1999**, *4*, 901-910.

[117] Deng, Y.; Gao, G.; He, Z.; Kispert, L.D. *J. Phys. Chem. B*, **2000**, *104*, 5651-5656.

[118] Dawe, E.A.; Land, E.J. *J. Chem. Soc. Faraday Trans. 1*, **1975**, *71*, 2162-2169.

[119] Lupinski, J.H. *J. Phys. Chem.*, **1963**, *67*, 2725-2728.

[120] Getoff, N. *Radiat. Res.*, **2000**, *154*, 692-696.

[121] Bally, T.; Roth, K.; Tang, W.; Schrock, R.R.; Knoll, K.; Park, L.Y. *J. Am. Chem. Soc.*, **1992**, *114*, 2440-2446.

[122] Poole, C.P.; Farach, H.A. Handbook of electron spin resonance : data sources, computer technology, relaxation, and ENDOR. AIP Press, New York, **1994**.

[123] Gao, Y.; Konovalova, T.A.; Xu, T.; Kispert, L.D. *J. Phys. Chem. B*, **2002**, *106*, 10808-10815.

[124] Konovalova, T.A.; Krzystek, J.; Bratt, P.J.; Van Tol, J.; Brunel, L.-C.; Kispert, L.D. *J. Phys. Chem. B*, **1999**, *103*, 5782-5786.

[125] Piekara-Sady, L.; Kispert, L.D. *Mol. Phys. Rep.*, **1994**, *6*, 220-223.

[126] Konovalova, T.A.; Kispert, L.D.; Konovalov, V.V. *J. Phys. Chem. B*, **1997**, *101*, 7858-7862.

[127] Jeevarajan, A.S.; Khaled, M.; Kispert, L.D. *J. Phys. Chem.*, **1994**, *98*, 7777-7781.

[128] Jeevarajan, A.S.; Khaled, M.; Kispert, L.D. *Chem. Phys. Lett.*, **1994**, *225*, 340-345.

[129] Piekara-Sady, L.; Jeevarajan, A.S.; Kispert, L.D. *Chem. Phys. Lett.*, **1993**, *207*, 173-177.

[130] Konovalova, T.A.; Kispert, L.D.; Polyakov, N.E.; Leshina, T.V. *Free Radic. Biol. Med.*, **2000**, *28*, 1030-1038.

[131] Polyakov, N.E.; Konovalov, V.V.; Leshina, T.V.; Luzina, O.A.; Salakhutdinov, N.F.; Konovalova, T.A.; Kispert, L.D. *J. Photochem. Photobiol. A. Chem.*, **2001**, *141*, 117-126.

[132] Bautista, J.A.; Chynwat, V.; Cua, A.; Jansen, F.J.; Lugtenburg, J.; Gosztola, D.; Wasielewski, M.R.; Frank, H.A. *Photosynth. Res.*, **1998**, *55*, 49-65.

[133] Konovalova, T.A.; Dikanov, S.A.; Bowman, M.K.; Kispert, L.D. *J. Phys. Chem. B*, **2001**, *105*, 8361-8368.

[134] Koyama, Y. In *Carotenoids Vol 1B: Spectroscopy*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1995**, pp. 135-146.

[135] Koyama, Y.; Mubai, Y. In *Advances in spectroscopy*; Clark, R.J.H.; Hester, R.E., Ed.; Wiley: Chichester, **1993**, Vol. *21*, pp. 49.

[136] Jeevarajan, A.S.; Kispert, L.D.; Chumanov, G.; Zhou, C.; Cotton, T.M. *Chem. Phys. Lett.*, **1996**, *259*, 515-522.

[137] Tinkler, J.H.; Tavender, S.M.; Parker, A.W.; McGarvey, D.J.; Mulroy, L.; Truscott, T.G. *J. Am. Chem. Soc.*, **1996**, *118*, 1756-1761.

[138] Polyakov, N.; Leshina, T.; Kispert, L. *RIKEN Rev.*, **2002**, *44*, 140-143.

[139] Broszeit, G.; Diepenbrock, F.; Graf, O.; Hecht, D.; Heinze, J.; Martin, H.D.; Mayer, B.; Schaper, K.; Smie, A.; Strehblow, H.H. *Liebigs Ann.*, **1997**, *11*, 2205-2213.

[140] Kispert, L.D.; Gao, G.; Deng, Y.; Konovalov, V.; Jeevarajan, A.S.; Jeevarajan, J.A.; Hand, E. *Acta Chem. Scand.*, **1997**, *51*, 572-578.

[141] Kawashima, Y.; Nakayama, K.; Nakano, H.; Hirao, K. *Chem. Phys. Lett.*, **1997**, *267*, 82-90.

[142] Khaled, M.; Hadjipetrou, A.; Kispert, L.D.; Allendoerfer, R.D. *J. Phys. Chem.*, **1991**, *95*, 2438-2442.

[143] Jeevarajan, A.S.; Wei, C.C.; Kispert, L.D. *J. Chem. Soc., Perkin Trans. 2*, **1994**, *4*, 861-869.

[144] Krawczyk, S. *Chem. Phys.*, **1998**, *230*, 297-304.

[145] Khaled, M.; Hadjipetrou, A.; Kispert, L. *J. Phys. Chem.*, **1990**, *94*, 5164-5169.

[146] Szabolcs, J.; Ronai, A. *Acta Chim. Acad. Sci. Hung.*, **1969**, *61*, 301-308.

[147] Eugster, C.H. In *Carotenoids Vol 1A: Isolation and Analysis*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Ed.; Birkhäuser: Basel, **1995**, Vol. *1A*, pp. 71-80.

[148] Maerki-Fischer, E.; Buchecker, R.; Eugster, C.H. *Helv. Chim. Acta*, **1984**, *67*, 461-466.

[149] Sorensen, T.S. *J. Am. Chem. Soc.*, **1965**, *87*, 5075-5084.

[150] Lutnaes, B.F.; Krane, J.; Liaaen-Jensen, S. In *Book of Abstracts, 13th Int. Carotenoid Symp.* Honolulu, **2002**, p. 108.

[151] Lafferty, J.; Roach, A.C.; Sinclair, R.S.; Truscott, T.G.; Land, E.J. *J. Chem. Soc. Faraday Trans. 1*, **1977**, *73*, 416-429.

[152] Getoff, N. *Radiat. Physics Chem.*, **1999**, *55*, 395-398.

[153] Stafstroem, S.; Bredas, J.L.; Loegdlund, M.; Salaneck, W.R. *J. Chem. Phys.*, **1993**, *99*, 7938-7945.

[154] Furukawa, Y.; Uchida, Y.; Tasumi, M.; Spangler, C.W. *Mol. Cryst. Liq. Cryst. Sci. Technol. Sect. A*, **1994**, *256*, 721-726.

[155] Hoitjink, G.J.; van der Meij, P.H. *Z. Phys. Chem.*, **1959**, *20*, 1-14.

[156] Kim, J.-Y.; Furukawa, Y.; Tasumi, M. *Chem. Phys. Lett.*, **1997**, *276*, 418-422.

[157] Ioffe, N.T.; Engovatov, A.A.; Mairanovskii, V.G. *Zh. Obshch. Khim.*, **1975**, *45*, 951-952.

[158] Grutzner, J.B. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D.M.; Harris, R.K., Ed.; Wiley: Chichester, **2002**, Vol. *9*, pp. 481-497.

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# BIOACTIVE CONSTITUENTS OF THE GENUS *HERNANDIA*

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**ABSTRACT:** Plants in the genus *Hernandia* of the family Hernandiaceae have been used ethnobotanically to treat malaria, postpartum hemorrhage, and morning sickness. Additionally, crude extracts of *H. moerenhoutiana* and *H. voyronii* have been reported to exhibit certain antioxidant, antimalarial, cytotoxic, and spasmolytic activities, as well as to inhibit platelet aggregation and prostaglandin biosynthesis. Of the approximately 24 species in this genus, about a quarter have been subjected to detailed phytochemical investigation, namely, *H. cordigera*, *H. guianensis*, *H. ovigera*, *H. ovigera* var. *mascarenensis*, *H. peltata* (*H. nymphaeifolia*), *H. sonora*, and *H. voyronii*. From over 130 compounds obtained from these species, the major secondary metabolites are represented by various classes of isoquinoline alkaloids and lignans, along with minor constituents of other alkaloidal (isoindole and pyridine) types, as well as benzenoids, flavonoids, monoterpenoids, and sterols. Some of these isolates have been found to exhibit various biological activities, such as antiplasmodial action, antiplatelet aggregation, cytotoxicity, inhibition of Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol 13-acetate, and inhibition of the transformation of JB6 mouse epidermal cells. The biologically active constituents of the genus *Hernandia* are discussed in detail.

## INTRODUCTION

The genus *Hernandia* L. of the family Hernandiaceae is represented by evergreen trees and shrubs [1]. *Hernandia* consists of approximately 24 species and is widely distributed in pantropical regions [2]. In terms of their economic applications, timbers of some species of *Hernandia* are used locally for crates, boxes, and cheap plywood [3]. Thus far, six species and one variety have been investigated for their secondary

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metabolites, namely, *H. cordigera* Vieill., *H. guianensis* Aubl., *H. ovigera* L., *H. ovigera* L. var. *mascarenensis* Meisn. [*H. mascarenensis* (Meisn.) Kubitzki], *H. peltata* Meisn. [*H. nymphaeifolia* (Presl.) Kubitzki], *H. sonora* L., and *H. voyronii* Jum. Several types of bioactive constituents (e.g., isoquinoline alkaloids and lignans) isolated from the genus *Hernandia* have been reported to exhibit various biological activities, such as antiplatelet aggregation [4], cytotoxicity [5], inhibition of Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol 13-acetate [6], and inhibition of the transformation of JB6 mouse epidermal cells [7]. Although a previous chapter in the book series, *Studies in Natural Products Chemistry*, contributed by Arimoto *et al.*, dealt with the structure elucidation and synthesis of the lignans of *Hernandia ovigera* specifically [8], the present chapter will review in turn the taxonomy, ethnobotanical uses, phytochemistry, and bioactive constituents of the whole genus *Hernandia*, up to the end of 2002.

## TAXONOMY OF THE GENUS *HERNANDIA*

According to Cronquist's classification system on flowering plants [2,9,10], the classification hierarchy of the genus *Hernandia* can be tracked as follows:

| | |
|---|---|
| Kingdom | Metaphyta |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Subclass | Magnoliidae |
| Order | Laurales |
| Family | Hernandiaceae |
| Subfamily | Hernandioideae |
| Genus | *Hernandia* |

The genus *Hernandia* L. is represented in pantropical regions and consists of 24 species [2], inclusive of *H. albiflora* (C.T. White) Kubitzki, *H. beninensis* Welw. ex Henriq., *H. bivalves* Benth., *H. catalpifolia* Britton & Fawc., *H. cordigera*, *H. cubensis* Griseb., *H. didymantha* Donn. Sm., *H. drakeana* Nadeaud, *H. guianensis*, *H. jamaicensis* Britton & Fawc., *H. mascarenensis*, *H. moerenhoutiana* Guillem., *H. nukuhivensis*

F. Br., *H. obovata* O.C. Schmidt, *H. olivacea* Gillespie, *H. ovigera*, *H. peltata*, *H. rostrata* Kubitzki, *H. sonora*, *H. stenura* Standl., *H. stokesii* (F. Br.) Kubitzki, *H. tahitensis* Moore, *H. temarii* Nadeaud, and *H. voyroni*.

The taxonomic characterization of *Hernandia* was described by Verdcourt [1] as follows:

> Trees or rarely shrubs, almost always evergreen, monoecious or rarely dioecious. Leaves simple, rarely 3–5-lobed, sometimes peltate in the juvenile state with pinnate or palmate nervation. Inflorescences usually aggregated at the apices of branches, with well-developed or rarely short peduncles; bracts present; partial inflorescences (1–)3-flowered with 1–2 lower pedicellate ♂ flowers and an upper subsessile ♀ flower, usually tomentellous, rarely glabrous; bracteoles of ♂ flower ± equal, approximate, including the partial inflorescences in bud; bracteoles of ♀ flower connate into a cupule which envelops the ovary and later the fruit; rarely free. Outer perianth-segments with imbricate or quincuncial aestivation; internal ones imbricate to valvate. Male flowers 3–5(–6)-merous, only rarely with a rudimentary style, mostly with both ovary and style wanting; stamens 3–5(–6), the filaments with 2 appendages which are sometimes joined. Female flowers 4–6-merous; ovary slightly laterally compressed, surrounded by 4–5(–12) free or connate glands; style straight to S-shaped, slightly thickened towards the base; true staminodes wanting. Drupes ovoid or elongate-ellipsoid, usually glabrous, at maturity enclosed in a cupule or rarely within free bracteoles. Cotyledons free, ± thick or joined and ruminate.

## ETHNOBOTANICAL AND BIOLOGICAL STUDIES ON PLANTS IN THE GENUS *HERNANDIA*

Three *Hernandia* species, namely, *H. moerenhoutiana*, *H. nymphaeifolia* (a synonym of *H. peltata* according to Kubitzki [2]), and *H. voyronii*, have been used ethnobotanically by inhabitants of Tonga and Madagascar to treat malaria [11,12], postpartum hemorrhage [13], retained placenta [13], and morning sickness [14].

Crude extracts of *H. moerenhoutiana* and *H. voyronii* were found to exhibit certain antioxidant, antimalarial, cytotoxic, and spasmolytic activities, as well as to inhibit platelet aggregation and prostaglandin biosynthesis [4,15-18], as summarized in Table 1.

**Table 1. Summary of Reported Biological Activities of *Hernandia* Species**

| Species | Part used | Extract | Biological activities | Reference(s) |
|---|---|---|---|---|
| *H. nymphaeifolia*[a] | Bark | Ethanol | Weakly inhibitory: prostaglandin biosynthesis | [15] |
| | | Not specified | Spasmolytic: guinea pig ileum | [16] |
| | Leaf | Methanol | Antioxidant | [17] |
| | Trunk bark | Methanol | Inhibitory: platelet aggregation | [4] |
| *H. voyronii* | Stem bark | Alkaloid fraction[b] | Antimalarial: *Plasmodium falciparum* (chloroquine resistant) Weakly cytotoxic: HeLa cells (uterine carcinoma), L929 transformed fibroblasts | [18] |

[a]Synonym of *Hernandia peltata* Meisn. [2].
[b]A polar alkaloidal fraction collected from a silica gel chromatographic column when eluted with isopropanol-water (7:3).

## PREVIOUS PHYTOCHEMICAL INVESTIGATIONS ON *HERNANDIA* SPECIES

A thorough literature search has indicated that six species and one variety of *Hernandia* have been well studied for their secondary metabolites, namely, *H. cordigera*, *H. guianensis*, *H. ovigera*, *H. ovigera* var. *mascarenensis*, *H. peltata* (*H. nymphaeifolia*), *H. sonora*, and *H. voyronii*, as summarized in Table 2. Previous phytochemical investigations on *Hernandia* species have resulted mainly in the isolation of various classes of isoquinoline alkaloids and lignans, along with minor constituents of other alkaloidal (isoindole and pyridine) types, as well as benzenoids, flavonoids, monoterpenoids, and sterols. The chemical structures of these isolates from the genus *Hernandia* are shown in Fig. (**1**).

## IDENTIFICATION AND STRUCTURE ELUCIDATION OF REPRESENTATIVE CONSTITUENTS OF *HERNANDIA* SPECIES

In the following paragraphs, brief details of the identification and structure elucidation of ten selected compounds will be provided, which represent seven types of isoquinoline alkaloids and three types of lignans. Representative isoquinoline alkaloids selected include a *p*-quinonoid

## Table 2. Known Constituents of Plants in the Genus *Hernandia*

| Compound type/name | Plant species | Part used | Reference(s) |
|---|---|---|---|
| **ALKALOIDS** | | | |
| **Isoindole** | | | |
| 5,6-Dimethoxy-*N*-methylphthalimide (**1**) | *H. nymphaeifolia*[a] | Trunk bark | [19] |
| **Isoquinoline** | | | |
| (+)-Actinodaphnine (**2**) | *H. cordigera*<br>*H. guianensis*<br>*H. ovigera* var. *mascarenensis*[b] | Leaf<br>Leaf<br>Leaf | [20]<br>[21]<br>[22] |
| Atheroline (**3**) | *H. nymphaeifolia*[a]<br>*H. sonora* | Trunk bark<br>Root bark | [4]<br>[23] |
| Backebergine (**4**) | *H. sonora* | Root bark | [23] |
| (+)-Corytuberine (**5**) | *H. sonora* | Root bark | [23] |
| Dehydrohernandaline (**6**) | *H. nymphaeifolia*[a]<br>*H. sonora* | Trunk bark<br>Root bark | [4]<br>[23] |
| Demethylsonodione (**7**) | *H. sonora* | Stem bark | [24] |
| 6,7-Dimethoxy-*N*-methylisoquinoline (**8**) | *H. ovigera* | Not specified | [25] |
| 7-Formyldehydrohernangerine (**9**) | *H. nymphaeifolia*[a] | Trunk bark | [19] |
| 7-Formyldehydronornantenine (**10**) | *H. sonora* | Root bark | [23] |
| 7-Formyldehydroovigerin (**11**) | *H. sonora* | Root bark | [23] |
| (+)-*N*-Formyldehydroovigerine (**12**) | *H. nymphaeifolia*[a] | Trunk bark | [5] |
| *N*-Formylhernangerine (**13**) | *H. nymphaeifolia*[a] | Trunk bark | [26] |
| (+)-*N*-Formylnornantenine (**14**) | *H. nymphaeifolia*[a] | Trunk bark | [26] |
| (+)-*N*-Formylovigerine (**15**) | *H. nymphaeifolia*[a] | Trunk bark | [26] |
| (+)-Hernagine (**16**) | *H. cordigera*<br>*H. nymphaeifolia*[a]<br>*H. ovigera* var. *mascarenensis*[b] | Stem bark<br>Leaf<br>Stem bark | [27,28]<br>[29]<br>[22] |
| (+)-Hernandaline (**17**) | *H. nymphaeifolia*[a] | Trunk bark | [4,30] |

**Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)**

| Compound type/name | Plant species | Part used | Reference(s) |
|---|---|---|---|
| Hernandonine (**18**) | *H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. ovigera* var. *mascarenensis*[b]<br>*H. sonora* | Trunk/stem bark<br>Root bark<br>Leaf<br><br>Root bark | [2,19,31]<br>[32-34]<br>[22]<br><br>[23] |
| Hernangerine (**19**) | *H. sonora* | Root bark | [23] |
| Hernanymphine (**20**) | *H. nymphaeifolia*[a] | Stem bark | [31] |
| (+)-Hernovine (**21**) | *H. guianensis*<br>*H. nymphaeifolia*[a]<br>*H. ovigera* var. *mascarenensis*[b] | Stem/root bark<br>Trunk bark<br>Stem bark | [21]<br>[5]<br>[22] |
| 7-Hydroxy-6-methoxy-1-methylisoquinoline (**22**) | *H. nymphaeifolia*[a] | Trunk bark | [19] |
| (+)-*N*-Hydroxyhernangerine (**23**) | *H. nymphaeifolia*[a] | Trunk bark | [5] |
| *N*-Hydroxyovigerine (**24**) | *H. nymphaeifolia*[a] | Trunk bark | [26] |
| (+)-Isoboldine (**25**) | *H. cordigera* | Leaf | [20] |
| (+)-Isocorydine (**26**) | *H. cordigera*<br>*H. nymphaeifolia*[a] | Stem bark<br>Leaf | [27,28]<br>[29] |
| Laetanine (**27**) | *H. voyronii* | Stem bark | [12] |
| (–)-Laetine (**28**) | *H. peltata* | Trunk bark | [35] |
| Laudanosine (**29**) | *H. voyronii* | Stem bark | [12] |
| (+)-Laurotetanine (**30**) | *H. cordigera*<br>*H. guianensis*<br>*H. nymphaeifolia*[a] | Stem bark<br>Leaf, stem bark<br>Leaf, trunk bark | [27,28]<br>[21]<br>[4,29] |
| Lindcarpine (**31**) | *H. voyronii* | Stem bark | [12] |
| (+)-Magnoflorine (**32**) | *H. nymphaeifolia*[a] | Trunk bark | [5] |
| (+)-*N*-Methylactinodaphnine (**33**) | *H. cordigera* | Leaf | [28] |
| *N*-(*N*-Methylcarbamoyl)-*O*-methylbulbocapnine (**34**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |
| *N*-Methylcorydaldine (**35**) | *H. nymphaeifolia*[a]<br>*H. ovigera* | Trunk bark<br>Not specified, twig | [30]<br>[7,25] |
| (+)-*N*-Methyl-6,7-dimethoxyisoquinolone (**36**) | *H. sonora* | Root bark | [23] |

**Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)**

| **Compound type/name** | **Plant species** | **Part used** | **Reference(s)** |
|---|---|---|---|
| *N*-Methylhernangerine (**37**) | *H. sonora* | Root bark | [23] |
| *N*-Methylhernovine (**38**) | *H. guianensis*<br>*H. nymphaeifolia*[a] | Stem bark<br>Trunk bark | [21]<br>[5] |
| (+)-*N*-Methyllaurotetanine (**39**) | *H. cordigera*<br>*H. nymphaeifolia*[a]<br>*H. voyronii* | Leaf<br>Leaf<br>Stem bark | [20]<br>[29]<br>[12] |
| *N*-Methylnandigerine (**40**) | *H. guianensis*<br>*H. ovigera* var. *mascarenensis*[b] | Leaf, stem bark<br>Stem bark | [21]<br>[22] |
| (+)-*N*-Methylovigerine (**41**) | *H. nymphaeifolia*[a] | Trunk bark | [30] |
| (+)-Nandigerine (hernangerine, **42**) | *H. cordigera*<br>*H. guianensis*<br>*H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. ovigera* var. *mascarenensis*[b] | Stem bark<br>Leaf, stem bark<br>Leaf<br>Root bark<br>Leaf, stem bark | [27,28]<br>[21]<br>[29]<br>[33,34]<br>[22] |
| (+)-Neolitsine (**43**) | *H. cordigera* | Leaf | [20] |
| (+)-Norisocorydine (**44**) | *H. cordigera*<br>*H. voyronii* | Stem bark<br>Stem bark | [27,28]<br>[11] |
| (+)-Nornantenine (**45**) | *H. cordigera*<br>*H. nymphaeifolia*[a] | Leaf, stem bark<br>Leaf | [20,27,28]<br>[29] |
| Norpredicentrine (**46**) | *H. voyronii* | Stem bark | [12] |
| Norsonodione (**47**) | *H. sonora* | Stem bark | [24] |
| Northalifoline (**48**) | *H. nymphaeifolia*[a] | Trunk bark | [5] |
| Ocobotrine (**49**) | *H. voyronii* | Stem bark | [11,12] |
| (+)-Ovigerine (**50**) | *H. cordigera*<br>*H. guianensis*<br>*H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. sonora* | Stem bark<br>Leaf, stem bark<br>Leaf<br>Root bark<br>Root bark | [27,28]<br>[21]<br>[29]<br>[33,34]<br>[23] |
| Oxocrebanine (**51**) | *H. ovigera* | Root bark | [33] |
| 7-Oxohernagine (**52**) | *H. nymphaeifolia*[a] | Stem/trunk bark | [4,31] |
| Oxohernandaline (**53**) | *H. nymphaeifolia*[a] | Trunk bark | [30] |
| 7-Oxohernangerine (**54**) | *H. nymphaeifolia*[a] | Stem/trunk bark | [4,31] |

## Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)

| Compound type/name | Plant species | Part used | Reference(s) |
|---|---|---|---|
| Oxonantenine (**55**) | *H. nymphaeifolia*[a] | Leaf | [29] |
| (–)-Pallidine (**56**) | *H. voyronii* | Stem bark | [12] |
| (+)-Reticuline [**57**, (*S*)-reticuline] | *H. cordigera*<br>*H. guianensis*<br>*H. nymphaeifolia*[a]<br>*H. peltata*<br>*H. voyronii* | Leaf, stem bark<br>Leaf, stem bark<br>Trunk bark<br>Bark<br>Stem bark | [20,27,36]<br>[21]<br>[4,5]<br>[37]<br>[12] |
| Sonodione (**58**) | *H. sonora* | Stem bark | [24] |
| Thalifoline (**59**) | *H. nymphaeifolia*[a] | Trunk bark | [5] |
| Xanthoplanine (**60**) | *H. ovigera* | Stem | [33] |
| **Isoquinoline dimer** | | | |
| (+)-Ambrimine (**61**) | *H. peltata* | Trunk bark | [35,38] |
| Dehydrothalicarpine (**62**) | *H. ovigera* | Root bark | [33,34] |
| 6a,7-Dehydrothalmelatine (**63**) | *H. peltata* | Trunk bark | [35] |
| (+)-Efatine (**64**) | *H. peltata* | Trunk bark | [35,38] |
| (+)-Hebridamine (**65**) | *H. peltata* | Trunk bark | [35] |
| Herveline A (**66**) | *H. voyronii* | Entire plant<br>Stem bark | [39]<br>[11,12] |
| Herveline B (**67**) | *H. voyronii* | Stem bark | [11,12] |
| Herveline C (**68**) | *H. voyronii* | Entire plant<br>Stem bark | [39]<br>[11,12] |
| Herveline D (**69**) | *H. voyronii* | Stem bark | [12] |
| (+)-Malekulatine (**70**) | *H. peltata*<br>*H. sonora* | Bark, trunk bark<br>Root bark | [35,37]<br>[23] |
| (+)-6-Northalicarpine (**71**) | *H. peltata* | Trunk bark | [35] |
| Ovigeridimerine (**72**) | *H. nymphaeifolia*[a] | Trunk bark | [4,19] |
| Ovihernangerine (**73**) | *H. nymphaeifolia*[a] | Trunk bark | [30] |

**Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)**

| Compound type/name | Plant species | Part used | Reference(s) |
|---|---|---|---|
| Oviisocorydine (**74**) | *H. nymphaeifolia*[a] | Trunk bark | [30] |
| Oxothalicarpine (**75**) | *H. ovigera* | Root bark | [34] |
| (+)-Thalicarpine (**76**) | *H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. peltata* | Trunk bark<br>Root bark<br>Trunk bark | [4,30]<br>[33,34]<br>[35] |
| (+)-Thalicarpine-2'-*N*-oxide (**77**) | *H. peltata* | Trunk bark | [35] |
| (+)-Thalmelatine (**78**) | *H. peltata* | Trunk bark | [35] |
| (+)-Vanuatine (**79**) | *H. peltata* | Bark, trunk bark | [35,37] |
| Vateamine (**80**) | *H. peltata* | Bark | [37] |
| (+)-Vateamine-2'β-*N*-oxide (**81**) | *H. nymphaeifolia*[a] | Trunk bark | [4,19] |
| (+)-Vilaportine (**82**) | *H. peltata* | Trunk bark | [35] |
| **Pyridine** | | | |
| 3-Cyano-4-methoxypyridine (**83**) | *H. nymphaeifolia*[a] | Leaf | [29] |
| **BENZENOIDS** | | | |
| Hernandial (**84**) | *H. nymphaeifolia*[a] | Trunk bark | [19] |
| *p*-Hydroquinone (**85**) | *H. ovigera* | Seed | [40] |
| Isovanillin (**86**) | *H. sonora* | Stem bark | [24] |
| **FLAVANONES** | | | |
| Nymphaeol A (**87**) | *H. nymphaeifolia*[a] | Leaf | [41] |
| Nymphaeol B (**88**) | *H. nymphaeifolia*[a] | Leaf | [41] |
| Nymphaeol C (**89**) | *H. nymphaeifolia*[a] | Leaf | [41] |
| **LIGNANS** | | | |
| (7*R*,8*S*,7'*R*,8'*R*)-(+)-7'-Acetyl-5'-methoxypicropodophyllin (**90**) | *H. ovigera* | Twig | [7] |

**Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)**

| **Compound type/name** | **Plant species** | **Part used** | **Reference(s)** |
|---|---|---|---|
| (7*R*,8*S*,7'*R*,8'*R*)-(+)-7'-Acetylpicropodophyllin (**91**) | *H. ovigera* | Twig | [7] |
| Acetylpodophyllotoxin (**92**) | *H. guianensis*<br>*H. sonora* | Stem/root bark<br>Seed | [21]<br>[42] |
| Anthricin (**93**) | *H. cordigera*<br>*H. guianensis*<br>*H. ovigera*<br>*H. sonora* | Leaf, stem<br>Leaf, stem bark<br>Seed, root bark<br>Seed | [43]<br>[21]<br>[33,44-47]<br>[48] |
| Bursehernin (**94**) | *H. cordigera*<br>*H. guianensis*<br>*H. ovigera*<br>*H. ovigera* var. *mascarenensis*[b]<br>*H. peltata* | Seed<br>Stem bark<br>Seed<br>Stem bark<br>Stem | [43]<br>[21]<br>[40,44-47]<br>[22]<br>[43] |
| Caruilignan C (**95**) | *H. ovigera* | Twig | [7] |
| Cordigerin (**96**) | *H. cordigera* | Stem | [43] |
| 1,2,3,4-Dehydrodeoxypodophyllotoxin (**97**) | *H. ovigera* | Seed | [6,40,46,49] |
| 1,2,3,4-Dehydropodophyllotoxin (**98**) | *H. ovigera* | Seed | [6,40,46] |
| 6,7-Demethylenedeoxypodophyllotoxin (**99**) | *H. ovigera* | Seed | [6,40] |
| Deoxypicropodophyllin (**100**) | *H. ovigera* | Seed | [44-46] |
| (–)-Deoxypodophyllotoxin (**101**) | *H. nymphaeifolia*[a]<br>*H. ovigera* | Trunk bark<br>Seed | [30]<br>[47] |
| Diasesartemin (**102**) | *H. ovigera* | Twig | [7] |
| Diayangambin (**103**) | *H. ovigera* | Twig | [7] |
| (–)-Dimethylmatairesinol (**104**) | *H. ovigera* | Seed | [47] |
| (+)-Epiaschantin (**105**) | *H. nymphaeifolia*[a]<br>*H. peltata* | Bark, trunk bark<br>Bark, leaf, stem | [4,30,50]<br>[43,50] |
| Epieudesmin (**106**) | *H. peltata* | Leaf, stem | [43] |
| (+)-Epimagnolin (**107**) | *H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. ovigera* var. *mascarenensis*[b]<br>*H. peltata* | Bark, trunk bark<br>Seed<br>Stem bark<br>Bark, leaf, stem | [4,31,50]<br>[6,40]<br>[22]<br>[43,50] |
| (+)-Epimagnolin A (**108**) | *H. ovigera* | Twig | [7] |

Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)

| Compound type/name | Plant species | Part used | Reference(s) |
|---|---|---|---|
| (+)-Epiyangambin (**109**) | *H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. peltata* | Trunk bark<br>Twig<br>Stem | [4,30]<br>[7]<br>[43] |
| Hernandin (**110**) | *H. cordigera*<br>*H. ovigera*<br>*H. sonora* | Stem<br>Seed<br>Seed | [43]<br>[46,47,49]<br>[48] |
| Hernolactone (**111**) | *H. ovigera* | Seed | [46,47] |
| (–)-Hernone (**112**) | *H. nymphaeifolia*[a] | Trunk bark | [4,51] |
| (–)-6'-Hydroxyyatein (**113**) | *H. nymphaeifolia*[a] | Trunk bark | [51] |
| 5-Methoxypodophyllotoxin (**114**) | *H. sonora* | Seed | [48] |
| 5-Methoxypodophyllotoxin acetate (**115**) | *H. sonora* | Seed | [48] |
| 5'-Methoxypodorhizol (**116**) | *H. cordigera*<br>*H. nymphaeifolia*[a]<br>*H. ovigera* | Leaf, stem (bark)<br>Trunk bark<br>Seed | [42,43]<br>[4]<br>[47] |
| 5'-Methoxypodorhizol acetate (**117**) | *H. sonora* | Seed | [48] |
| 5'-Methoxyyatein (**118**) | *H. cordigera*<br>*H. ovigera* | Leaf, seed, stem<br>Twig | [42,43]<br>[7] |
| (–)-Nymphone (**119**) | *H. nymphaeifolia*[a] | Trunk bark | [51] |
| Picropodophyllin (**120**) | *H. guianensis*<br>*H. sonora* | Bark<br>Seed | [52]<br>[48] |
| Podophyllotoxin (**121**) | *H. ovigera*<br>*H. sonora* | Seed<br>Seed | [40]<br>[48] |
| Podorhizol (**122**) | *H. cordigera*<br>*H. guianensis*<br>*H. ovigera* | Leaf, stem<br>Stem bark<br>Seed | [43]<br>[21]<br>[4,40,44-47] |
| (–)-Yatein (**123**) | *H. cordigera*<br>*H. nymphaeifolia*[a]<br>*H. ovigera*<br>*H. ovigera* var. *mascarenensis*[b]<br>*H. peltata* | Leaf, seed, stem<br>Bark, trunk bark<br>Seed, twig<br>Stem bark<br>Bark, stem | [42,43]<br>[4,30,50]<br>[7,46,47]<br>[22]<br>[43,50] |
| MONOTERPENE | | | |
| (+)-Perillaldehyde (**124**) | *H. voyronii* | Stem bark | [11] |
| STEROLS | | | |
| 3β-Hydroxystigmast-5,22-dien-7-one (**125**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |

**Table 2. Known Constituents of Plants in the Genus *Hernandia* (Continued)**

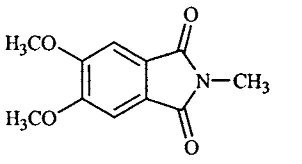
| Compound type/name | Plant species | Part used | Reference(s) |
|---|---|---|---|
| 6α-Hydroxystigmast-4,22-dien-3-one (**126**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |
| 3β-Hydroxystigmast-5-en-7-one (**127**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |
| 6α-Hydroxystigmast-4-en-3-one (**128**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |
| β-Sitosterol (**129**) | *H. guianensis* | Bark | [52] |
| β-Sitosterone (**130**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |
| Stigmasta-4,22-dien-3-one (**131**) | *H. nymphaeifolia*[a] | Trunk bark | [4] |
| Stigmasterol (**132**) | *H. guianensis* | Bark | [52] |

[a]Synonym of *H. peltata* [2].
[b]Synonym of *H. mascarenensis* [2].

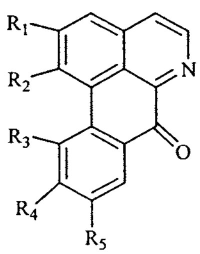
aporphine (**7**), a dehydroaporphine (**11**), a 1,2,10,11-tetrasubstituted aporphine (**15**), an oxoaporphine (**20**), a pavine-benzyltetraisoquinoline dimer (**66**), a bisbenzylisoquinoline (**70**), and a dimeric aporphine (**73**). The three lignans chosen are members of the aryltetralin lactone (**90**), furanoid (**112**), and dibenzylbutyrolactone types (**113**), respectively. The isolation and structure elucidation of **7**, **11**, **15**, **20**, **73**, **112**, and **113** were performed in the laboratory of Prof. Ih-Sheng Chen at Kaohsiung Medical College, Kaohsiung, Taiwan. The structure determination of compound **66** was carried out by Prof. Philippe Rasoanaivo *et al.* at Institut Malgache de Recherches Appliquées (IMRA), Antananarivo, Madagascar, compound **70** by Prof. Maurice Shamma *et al.* at Pennsylvania State University, and compound **90** by our group at the College of Pharmacy, University of Illinois at Chicago.

Demethylsonodione (**7**), a *p*-quinonoid aporphine, was obtained as greenish prisms. Its molecular formula was determined as $C_{19}H_{17}O_5N$ by high-resolution electron impact mass spectrometry (HREIMS) at *m/z* 339.1098 $[M]^+$ [24]. The UV spectrum with maxima at 219, 283, 336, and 642 nm was typical of a *p*-quinonoid aporphine skeleton [53]. In its IR spectrum, absorption bands at 3440 $cm^{-1}$ (phenolic hydroxyl) and 1635 $cm^{-1}$ (carbonyl) were apparent. The $^1H$-NMR spectrum indicated signals

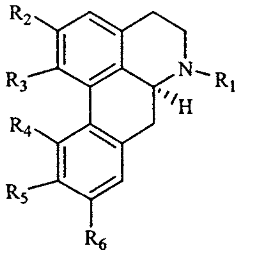
**Fig. (1).**



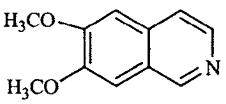

**1**



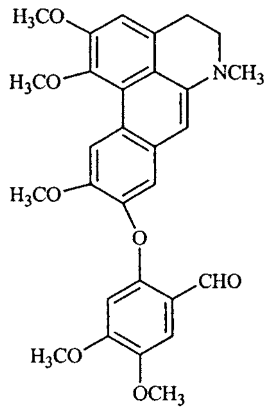

**3** $R_1=R_2=R_4=OCH_3$, $R_3=H$, $R_5=OH$
**18** $R_1+R_2=R_3+R_4=OCH_2O$, $R_5=H$
**20** $R_1=OCH_3$, $R_2=R_3=H$, $R_4+R_5=OCH_2O$
**52** $R_1=R_2=R_3=OCH_3$, $R_4=OH$, $R_5=H$
**54** $R_1+R_2=OCH_2O$, $R_3=OCH_3$, $R_4=OH$, $R_5=H$
**55** $R_1=R_2=OCH_3$, $R_3=H$, $R_4+R_5=OCH_2O$




**2** $R_1=R_4=H$, $R_2+R_3=OCH_2O$, $R_5=OCH_3$, $R_6=OH$
**5** $R_1=CH_3$, $R_2=R_5=OCH_3$, $R_3=R_4=OH$, $R_6=H$
**13** $R_1=CHO$, $R_2+R_3=R_4+R_5=OCH_2O$, $R_6=H$
**14** $R_1=CHO$, $R_2=R_3=OCH_3$, $R_4=H$, $R_5+R_6=OCH_2O$
**15** $R_1=CHO$, $R_2+R_3=R_4+R_5=OCH_2O$, $R_6=H$
**16** $R_1=R_6=H$, $R_2=R_3=R_4=OCH_3$, $R_5=OH$
**19** $R_1=H$, $R_2+R_3=OCH_2O$, $R_4=OCH_3$, $R_5=OH$, $R_6=H$
**21** $R_1=R_6=H$, $R_2=R_5=OH$, $R_3=R_4=OCH_3$
**23** $R_1=R_5=OH$, $R_2+R_3=OCH_2O$, $R_4=OCH_3$, $R_6=H$
**24** $R_1=OH$, $R_2+R_3=R_4+R_5=OCH_2O$, $R_6=H$
**25** $R_1=CH_3$, $R_2=R_5=OCH_3$, $R_3=R_6=OH$, $R_4=H$
**26** $R_1=CH_3$, $R_2=R_3=R_5=OCH_3$, $R_4=OH$, $R_6=H$
**27** $R_1=R_4=H$, $R_2=R_5=OH$, $R_3=R_6=OCH_3$
**28** $R_1=R_6=H$, $R_2=OH$, $R_3=OCH_3$, $R_4+R_5=OCH_2O$
**30** $R_1=R_4=H$, $R_2=R_3=R_5=OCH_3$, $R_6=OH$
**31** $R_1=R_6=H$, $R_2=R_4=OH$, $R_3=R_5=OCH_3$
**33** $R_1=CH_3$, $R_2+R_3=OCH_2O$, $R_4=H$, $R_5=OCH_3$, $R_6=OH$
**34** $R_1=CONHCH_3$, $R_2+R_3=OCH_2O$, $R_4=R_5=OCH_3$, $R_6=H$
**37** $R_1=CH_3$, $R_2+R_3=OCH_2O$, $R_4=OCH_3$, $R_5=OH$, $R_6=H$
**38** $R_1=CH_3$, $R_2=R_5=OH$, $R_3=R_4=OCH_3$, $R_6=H$
**39** $R_1=CH_3$, $R_2=R_3=R_5=OCH_3$, $R_4=H$, $R_6=OH$
**40** $R_1=CH_3$, $R_2+R_3=OCH_2O$, $R_4=OCH_3$, $R_5=OH$, $R_6=H$
**41** $R_1=CH_3$, $R_2+R_3=R_4+R_5=OCH_2O$, $R_6=H$
**42** $R_1=R_6=H$, $R_2+R_3=OCH_2O$, $R_4=OCH_3$, $R_5=OH$
**43** $R_1=CH_3$, $R_2+R_3=R_5+R_6=OCH_2O$, $R_4=H$
**44** $R_1=R_6=H$, $R_2=R_3=R_5=OCH_3$, $R_4=OH$
**45** $R_1=R_4=H$, $R_2=R_3=OCH_3$, $R_5+R_6=OCH_2O$
**46** $R_1=R_4=H$, $R_2=OH$, $R_3=R_5=R_6=OCH_3$
**50** $R_1=R_6=H$, $R_2+R_3=R_4+R_5=OCH_2O$

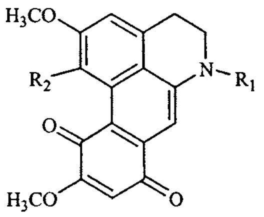



**4**

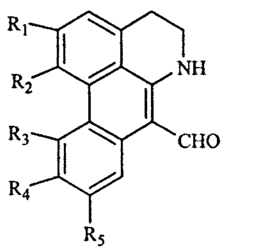



**6**

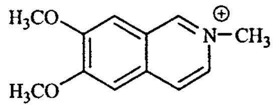
**Fig. (1).** Continued



**7** $R_1$=$CH_3$, $R_2$=OH
**47** $R_1$=H, $R_2$=$OCH_3$
**58** $R_1$=$CH_3$,$R_2$=$OCH_3$



**9** $R_1$+$R_2$=$OCH_2O$, $R_3$=$OCH_3$, $R_4$=OH, $R_5$=H
**10** $R_1$=$R_2$=$OCH_3$, $R_3$=H, $R_4$+$R_5$=$OCH_2O$
**11** $R_1$+$R_2$=$R_3$+$R_4$=$OCH_2O$, $R_5$=H



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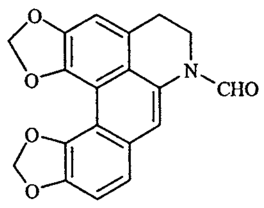

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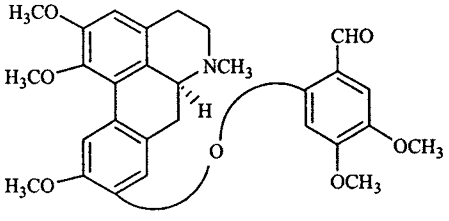
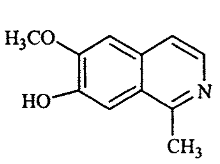


**17**



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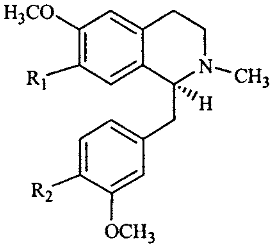
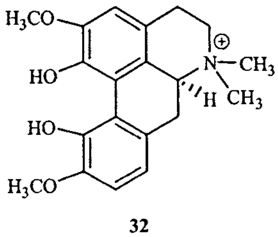
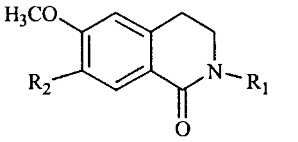


**29** $R_1$=$R_2$=$OCH_3$
**57** $R_1$=$R_2$=OH

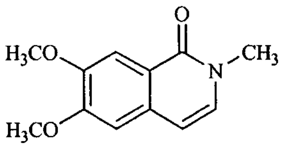


**32**

Fig. (1). Continued



35 $R_1$=$CH_3$, $R_2$=$OCH_3$
48 $R_1$=H, $R_2$=OH
59 $R_1$=$CH_3$, $R_2$=OH



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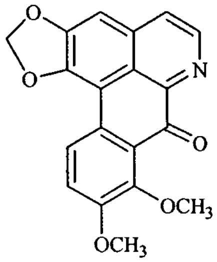
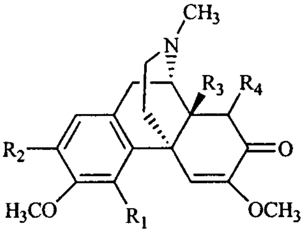
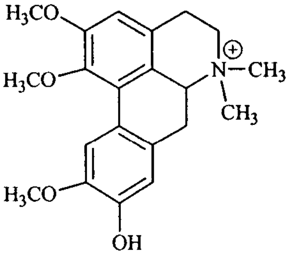


51



49 $R_1$=OH, $R_2$=$R_3$=$R_4$=H
56 $R_1$=H, $R_2$=OH, $R_3$+$R_4$=Δ



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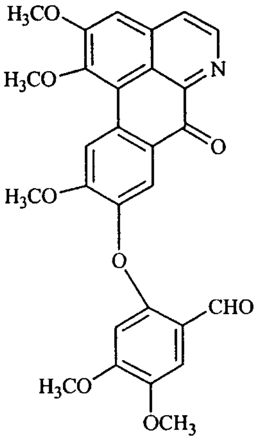

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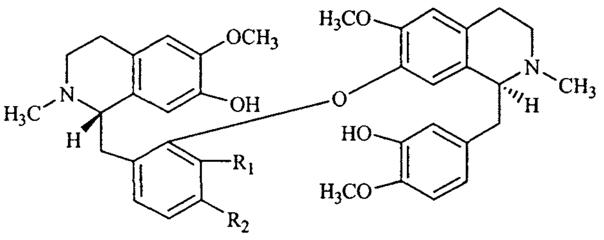
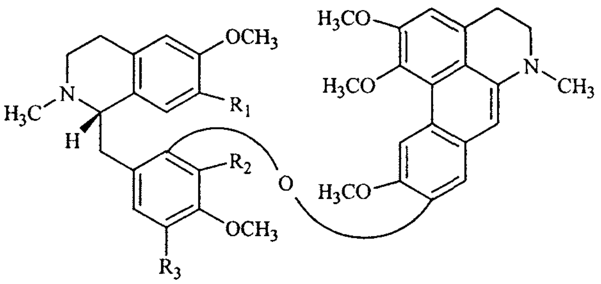
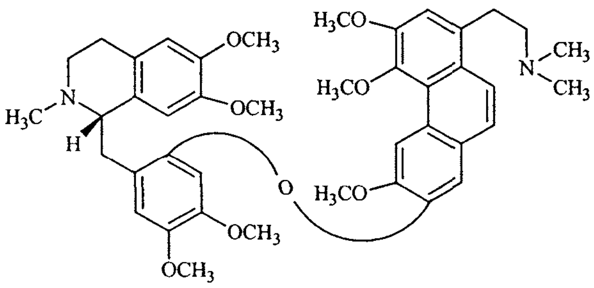


**61** $R_1$=$OCH_3$, $R_2$=OH
**64** $R_1$=OH, $R_2$=$OCH_3$

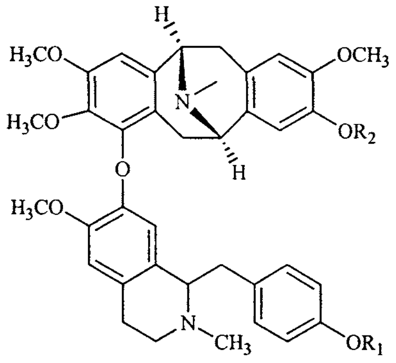


**62** $R_1$=$R_2$=$OCH_3$, $R_3$=H
**63** $R_1$=OH, $R_2$=H, $R_3$=$OCH_3$

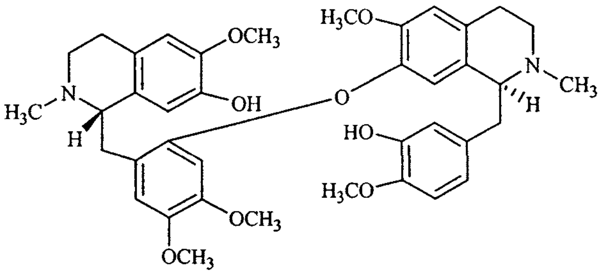


**65**

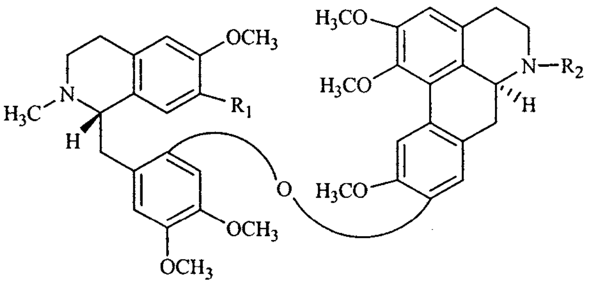
Fig. (1). Continued



**66** $R_1$=H, $R_2$=$CH_3$
**67** $R_1$=$CH_3$, $R_2$=H
**68** $R_1$=$R_2$=$CH_3$
**69** $R_1$=$R_2$=H

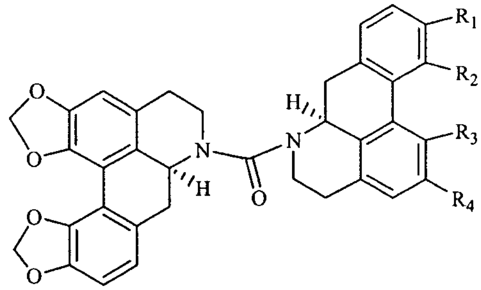


**70**

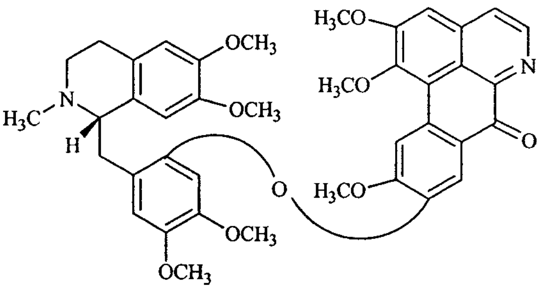


**71** $R_1$=$OCH_3$, $R_2$=H
**76** $R_1$=$OCH_3$, $R_2$=$CH_3$
**78** $R_1$=OH, $R_2$=$CH_3$

**Fig. (1).** Continued



**72** $R_1$+$R_2$= $R_3$+$R_4$=$OCH_2O$
**73** $R_1$=OH, $R_2$=$OCH_3$, $R_3$+$R_4$=$OCH_2O$
**74** $R_1$=$R_3$=$R_4$=$OCH_3$, $R_2$=OH



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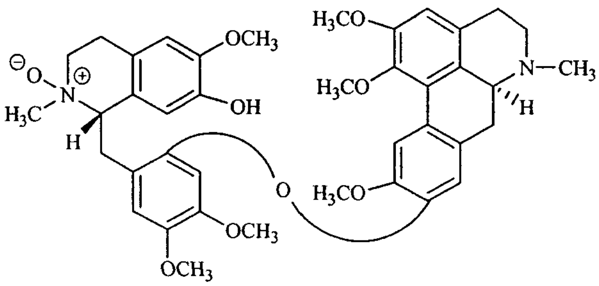
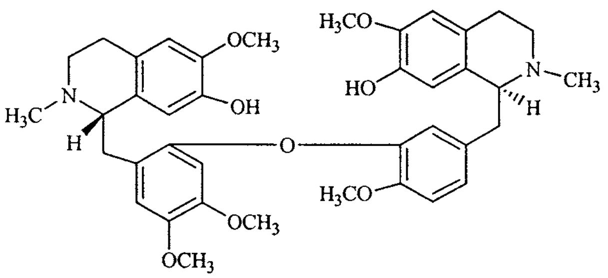


**77**

Fig. (1). Continued



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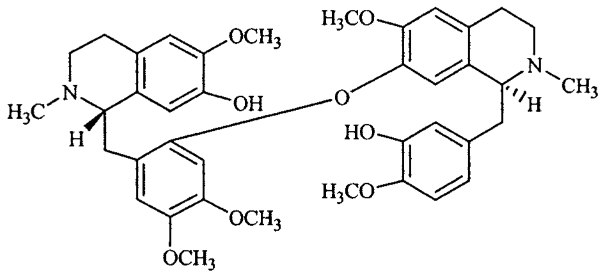

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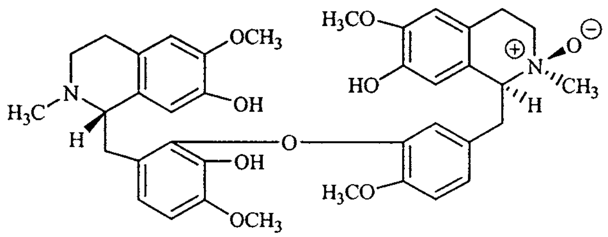

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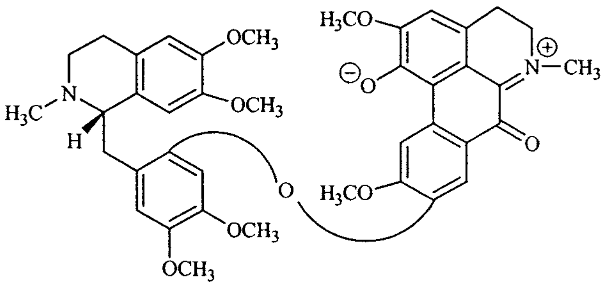

**82**

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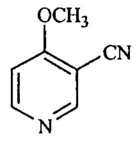

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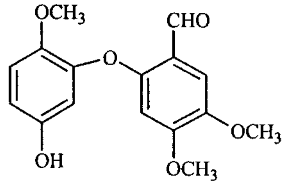

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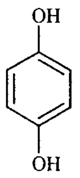

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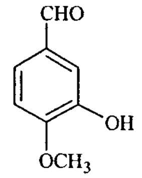

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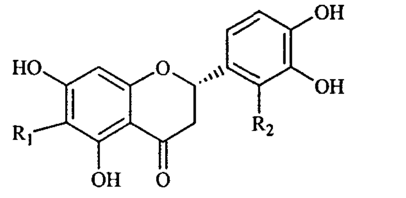
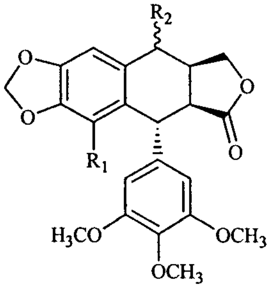
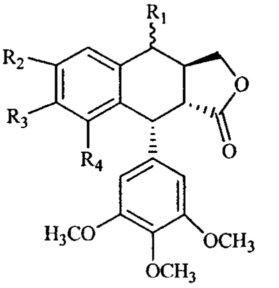


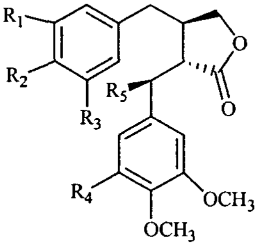
| | $R_1$ | $R_2$ |
| --- | --- | --- |
| 87 | (geranyl) | H |
| 88 | H | (geranyl) |
| 89 | (prenyl) | (geranyl) |



90 $R_1$ = $OCH_3$, $R_2$=αOAc
91 $R_1$ = H, $R_2$=αOAc
100 $R_1$=$R_2$=H
120 $R_1$ = H, $R_2$=αOH

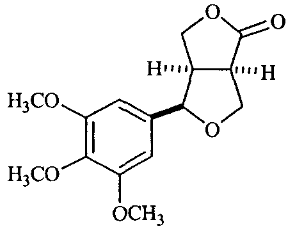


92 $R_1$=αOAc, $R_2$+$R_3$=$OCH_2O$, $R_4$=H
99 $R_1$=$R_4$=H, $R_2$+$R_3$=OH
101 $R_1$=$R_4$=H, $R_2$+$R_3$=$OCH_2O$
110 $R_1$=H, $R_2$+$R_3$=$OCH_2O$, $R_4$=$OCH_3$
121 $R_1$=αOH, $R_2$+$R_3$=$OCH_2O$, $R_4$=H

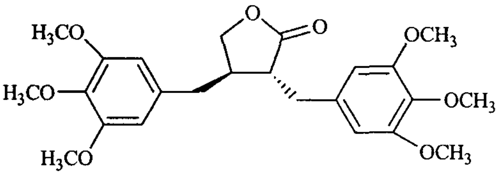


93 $R_1$=$R_2$=$R_3$=$R_4$=$OCH_3$, $R_5$=H
94 $R_1$+$R_2$=$OCH_2O$, $R_3$=$R_4$=$R_5$=H
104 $R_1$=$R_2$=$OCH_3$, $R_3$=$R_4$=$R_5$=H
111 $R_1$=OH, $R_2$=$R_3$=$R_4$=$OCH_3$, $R_5$=H
116 $R_1$+$R_2$=$OCH_2O$, $R_3$=$R_4$=$OCH_3$, $R_5$=OH
117 $R_1$+$R_2$=$OCH_2O$, $R_3$=$R_4$=$OCH_3$, $R_5$=OAc
118 $R_1$+$R_2$=$OCH_2O$, $R_3$=$R_4$=$OCH_3$, $R_5$=H
122 $R_1$+$R_2$=$OCH_2O$, $R_3$=H, $R_4$=$OCH_3$, $R_5$=OH
123 $R_1$+$R_2$=$OCH_2O$, $R_3$=$R_5$=H, $R_4$=$OCH_3$

Fig. (1). Continued



**95**



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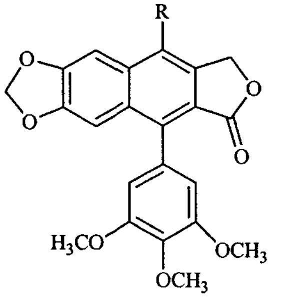
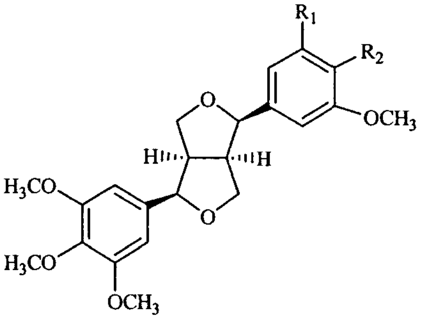
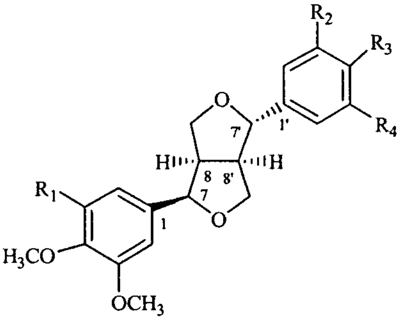


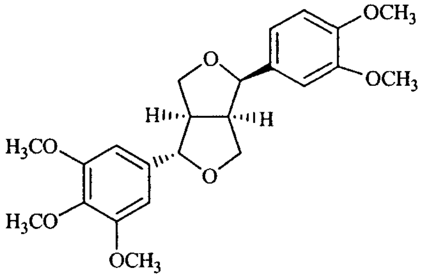
**97** R=H
**98** R=OH



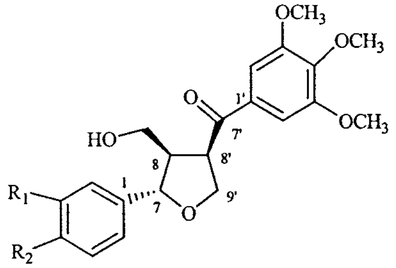
**102** $R_1+R_2=OCH_2O$
**103** $R_1=R_2=OCH_3$



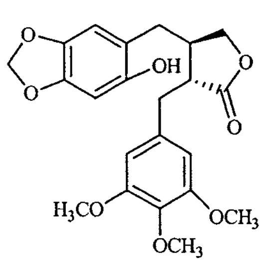
**105** $R_1=OCH_3$, $R_2+R_3=OCH_2O$, $R_4=H$
**106** $R_1=R_2=H$, $R_3=R_4=OCH_3$
**107** $R_1=R_2=R_3=OCH_3$, $R_4=H$
**109** $R_1=R_2=R_3=R_4=OCH_3$



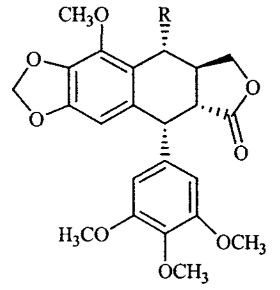
**108**



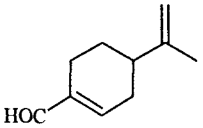
**112** $R_1=R_2=OCH_3$
**119** $R_1+R_2=OCH_2O$



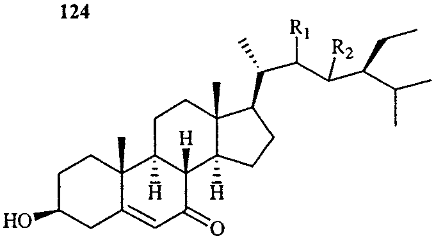
**113**



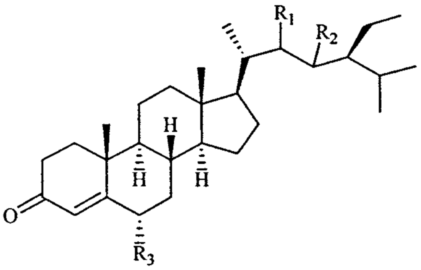
**114** R=OH
**115** R=OAc



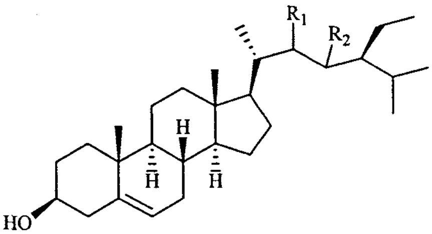
**124**



**125** $R_1+R_2=\Delta$
**127** $R_1=R_2=H$



**126** $R_1+R_2=\Delta$, $R_3=OH$
**128** $R_1=R_2=H$, $R_3=OH$
**130** $R_1=R_2=R_3=H$
**131** $R_1+R_2=\Delta$, $R_3=H$



**129** $R_1+R_2=\Delta$
**132** $R_1=R_2=H$

**Fig. (1).** Chemical structures of reported constituents of plants in the genus *Hernandia*.

for an *N*-methyl at $\delta_H$ 3.38, two methylenes at δ 3.18 ($H_2$-4) and 3.66 ($H_2$-5), two methoxyls at δ 3.92 ($OCH_3$-10) and 4.02 ($OCH_3$-2), three aromatic protons at δ 5.98 (H-9), 6.97 (H-3), and 7.18 (H-7), and one downfield hydroxyl at δ 11.4 (disappeared with $D_2O$, OH-1), due to the formation of hydrogen bonding with the neighboring C-11 carbonyl group [24]. All of the assignments were further confirmed by nuclear Overhauser enhancement (NOE) difference NMR experiments as shown in Fig. (**2**). The methoxyl signal at δ 3.92 was assigned to C-10 based on the absence of any 9-methoxyquinonoid aporphines in Nature and the close resemblance when compared with a similar synthetic analogue, sonodione (**58**) [24].

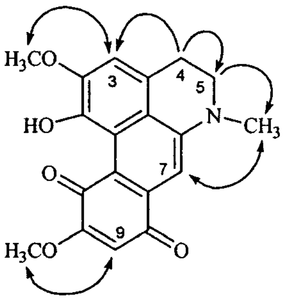



**Fig. (2).** NOE interactions observed for **7**.

7-Formyldehydroovigerine (**11**), a dehydroaporphine, was isolated as yellowish prisms, and its molecular formula was determined as $C_{19}H_{13}O_5N$ by HREIMS at *m/z* 335.0799 $[M]^+$ [23]. The UV absorptions at 234, 270, 295 sh, 345, and 429 nm were consistent with the presence of a dehydroaporphine skeleton [54]. The IR spectrum displayed a carbonyl band at 1625 $cm^{-1}$ and methylenedioxy bands at 1070 and 950 $cm^{-1}$ [23]. The $^1$H-NMR spectrum of **11** suggested two methylene units at δ 3.12 ($H_2$-4) and 3.62 ($H_2$-3), two methylenedioxy groups at δ 6.07 and 6.16, three aromatic protons at δ 6.92 (H-3), 7.08 (H-9), and 7.62 (H-8), a formyl group at δ 10.42, and a NH group at $\delta_H$ 11.40 (disappeared with $D_2O$) [23]. The formyl group was located at C-7 based on the observed downfield NH signal due to the formation of hydrogen-bonding between NH and the neighboring carbonyl group, along with nuclear Overhauser

enhancement (NOE) effects between H-8 and CHO and H-9, as shown in Fig. (**3**).

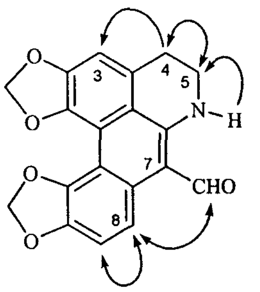
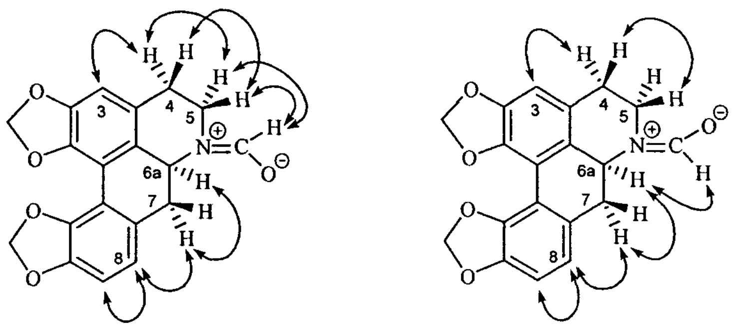



**Fig. (3).** NOE interactions observed for **11**.

(+)-*N*-Formylovigerine (**15**), $[\alpha]^{24}_D$ +321° (*c* 0.11, $CHCl_3$), was obtained as colorless prisms. The molecular formula was determined as $C_{19}H_{15}O_5N$ by HREIMS (obsd *m/z* 337.0959). The UV absorption bands at 227, 271, 278 sh, and 313 nm were suggestive of the presence of a 1,2,10,11-tetrasubstituted aporphine skeleton [33]. Its IR spectrum indicated that compound **15** contains amidocarbonyl (1660 $cm^{-1}$) and methylenedioxyl functional groups (1055 and 935 $cm^{-1}$) [26]. In its LREIMS, a base peak at *m/z* 279 suggested the presence of an *N*-formyl functionality due to loss of a moiety, $[CH_2\text{-N-CHO+H}]^+$, from the molecular ion. The $^1$H-NMR spectrum indicated that **15** exists the form of two rotational isomers (**15a** and **15b**) in a ratio of 2.5:1 due to the presence of the *N*-formyl group [26]. This rotational isomerism was fully supported by a NOESY experiment and evidenced by the proximity of the *N*-formyl proton to H-5β in the major isomer (**15a**) and to H-6a in the minor isomer (**15b**), as shown in Fig. (**4**).

Hernanymphine (**20**), an oxoaporphine, was obtained as yellowish prisms. Its molecular formula was established as $C_{18}H_{11}O_4N$ by HREIMS (obsd *m/z* 305.0688). The UV absorptions at 210, 241, 270, 292, 314 sh, 361, and 427 nm, along with the exhibition of a bathochromic shift in acid, indicated the presence of an oxoaporphine skeleton [55]. The IR spectrum exhibited a carbonyl at 1650 $cm^{-1}$ and a methylenedioxy group at 1030 and 960 $cm^{-1}$ [31]. Analysis of $^1$H-NMR spectrum of **20** led to the full assignments of a methoxyl at δ 4.04 ($OCH_3$-2), a methylenedioxy at δ

6.15, and six aromatic protons at δ 7.14 (H-3), 7.58 (H-11), 7.83 (H-4), 7.85 (H-1), 7.93 (H-8), and 8.92 (H-5). Its structure was further supported by NOE difference experiments, as shown in Fig. (5).



**15a** **15b**

**Fig. (4).** NOESY correlations for **15a** and **15b**.

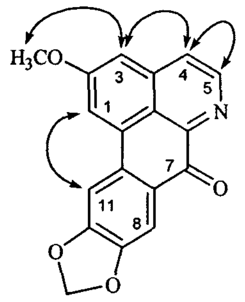


**Fig. (5).** NOE interactions observed for **20**.

Herveline A (**66**) is a pavine-benzyltetrahydroisoquinoline dimer, and its molecular formula was determined as $C_{39}H_{44}O_7N_2$ by HREIMS (obsd *m/z* 652.3140) [11]. The UV absorptions at 282 and 319 nm were indicative of a bisbenzylisoquinoline skeleton [37]. The $^1$H-NMR spectrum of **66** revealed the presence of two *N*-methyl groups, five methoxy groups, one $A_2B_2$ system, and five aromatic singlet protons [11]. In addition, three CH-$CH_2$ and one $CH_2$-$CH_2$ units were identified by $^1$H-

$^{1}H$ homonuclear correlation spectroscopy (COSY). The connection between the benzyltetraisoquinoline and pavine moieties in **66** was located at C-10 and C-7' through an ether bridge as a result of the unambiguous assignments of $^{1}H$ and $^{13}C$ NMR signals by the heteronuclear multiple-bond quantum coherence (HMQC) and heteronuclear multiple-bond coherence (HMBC) NMR techniques. The EIMS of **66** confirmed the presence of a hydroxybenzyl moiety due to the observed complementary peaks at *m/z* 545 and 107 [11]. Furthermore, the structure of **66** was substantiated by the formation of herveline C (**68**) after **66** was treated with diazomethane in ethyl ether solution overnight [11].

(+)-Malekulatine (**70**), a bisbenzylisoquinoline alkaloid, exhibited a weak molecular ion at *m/z* 670 in the low-resolution EIMS ($[M]^+$), which was consistent with a molecular formula of $C_{39}H_{46}O_8N_2$. The UV spectrum exhibited an absorption maximum at 284 nm, close to that of several tetrahydroisoquinolines [56,57]. Due to facile benzylic cleavage, the mass spectrum showed a base ion peak at *m/z* 192 which is generated from rings A and B, and an important peak at *m/z* 533 (8%) which corresponds to the loss of ring C' from the molecular ion [37]. This implied that compound **70** exhibited head-to-tail oxidative coupling. The $^{1}H$-NMR spectral assignments were confirmed by NOE difference measurements as summarized in Fig. (**6**). Furthermore, diazomethane *O*-methylation of **70** yielded (+)-*O,O*-dimethylmalekulatine (**70a**), $C_{41}H_{50}O_8N_2$, whose $^{1}H$-NMR spectrum showed an upfield *O*-methyl singlet at δ 3.50, diagnostic of a C-7 methoxyl of an *N*-methylated tetrahydrobenzylisoquinoline [37]. Finally, the absolute stereochemistry of **70** was determined with *S* configuration both at C-1 and C-1' due to the observed positive Cotton effects near 233 nm in the circular dichroism (CD) spectra of two tetrahydrobenzylisoquinolines (+)-**70b** and (–)-**70c**, which were produced by the cleavage of **70a** using metallic sodium in liquid ammonia [Fig. (**6**)] [37].

Ovihernangerine (**73**), a dimeric aporphine, was obtained as colorless prisms with a molecular formula of $C_{37}H_{30}O_9N_2$, which was determined by HREIMS at *m/z* 646.1959 ($[M]^+$). Its UV spectrum with maxima at 228, 272, and 311 nm was typical of 1,2,10,11-oxygenated aporphine skeleton [58]. The IR spectrum indicated that **73** contains a hydroxyl (3420 $cm^{-1}$), a carbonyl (1640 $cm^{-1}$), and a methylenedioxy group (1050 and 935 $cm^{-1}$) [30]. The important EIMS data at *m/z* 338 ([M-

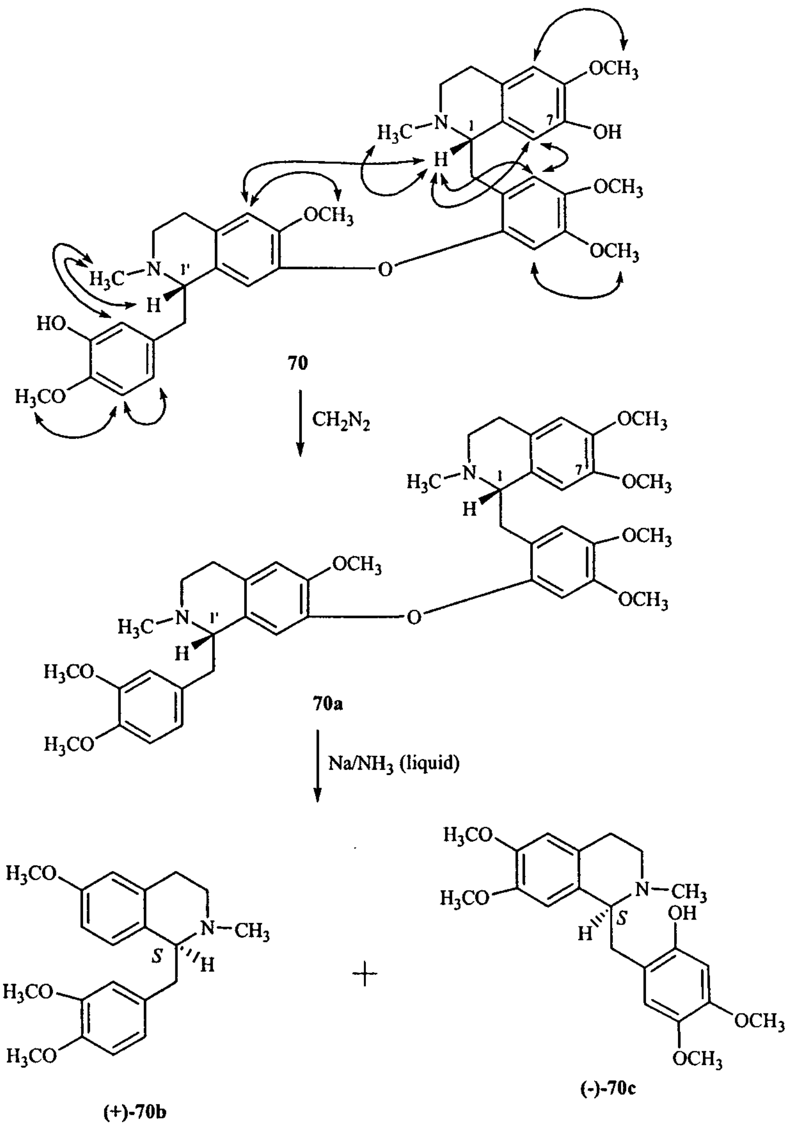



**Fig. (6).** NOE interactions observed for **70** and determination of absolute stereochemistry of **70** by evaluating its degradation products (+)-**70b** and (–)-**70c**.

$C_{18}H_{14}O_4N]^+$) and 308 ($[M-C_{18}H_{16}O_4N-CO]^+$) revealed that compound **73** is a dimer of hernangerine (**19**) and ovigerine (**50**) through a carbonyl linkage to connect two nitrogen atoms [30]. Comparison of the $^1$H-NMR

spectrum of **73** with those of **19** and **50** led to the full assignments of the proton signals. The absolute configurations at C-6a and C-6'a in **73** were both determined as *S* on the basis of the positive specific rotation value at +310° (*c* 0.08, $CHCl_3$), as in the case of ovigeridimerine (**72**) [62].

(7*R*,8*S*,7'*R*,8'*R*)-(+)-7'-Acetyl-5'-methoxypicropodophyllin (**90**), an aryltetralin lactone-type lignan, was obtained as a colorless resin with $[\alpha]^{20}_D$ +51.0° (*c* 0.069, MeCN) [7]. The molecular formula was determined as $C_{25}H_{26}O_{10}$ by HREIMS (obsd *m/z* 486.1547) [7]. Comparison of the $^1H$ and $^{13}C$ NMR spectral data obtained for **90** with those of 7'-acetyl-2'-methoxypicropodophyllin [59], indicated that they are a pair of isomers, with the only differences being in the disposition of methoxyl group. The methoxyl group in **90** was located at C-5' as a result of the observed HMBC correlation (H-2'/C-7'), and the ROESY correlation ($OCH_3$-5'/H-7), as shown in Fig. (**7**). The *cis* configuration of the fused lactone ring in **90** was determined on the basis of the coupling constant value of 9.2 Hz for the pair H-8/H-8', which was clearly different from the value reported for the *trans* configuration ($J$ = 14.8 Hz) [60]. The relative stereochemistry of **90** was determined to be H-7β, H-8α, H-7'β, and H-8'α as a result of the performance of a ROESY NMR experiment. The absolute configuration of the stereogenic center at the C-7 position in **90** was assigned as *R* from the positive Cotton effect at 287 nm in the circular dichroism (CD) spectrum [49], so the stereochemistry at C-8, C-7', and C-8' was deduced as *S*, *R*, and *R*, respectively [7].

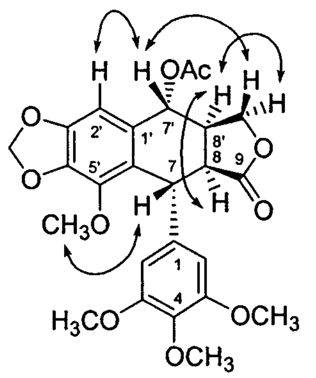



**Fig. (7).** Selected ROESY correlations observed for **90**.

(–)-Hernone (**112**), a furanoid lignan, was isolated as colorless prisms with $[\alpha]^{25}_{D}$ –29.7° (*c* 0.19, $CHCl_3$). Its molecular formula was determined to be $C_{23}H_{28}O_8$ by HREIMS at *m/z* 432.1782 ($[M]^+$). The UV absorptions at 204, 218 sh, 282 nm suggested the presence of a 2,3,4-trisubstituted furanoid lignan skeleton [61]. The IR spectrum of **112** revealed the presence of a conjugated carbonyl functionality (1660 $cm^{-1}$) and a hydroxyl group (3450 $cm^{-1}$). Analysis of the observed base peak at *m/z* 195 ($[ArCO]^+$) in the EIMS of **112** and two downfield equivalent aromatic protons at δ 7.27 in its $^1H$-NMR spectrum indicated the presence of an aroyl group substituted with three methoxyls in **112** [51]. In addition, a significant tropylium ion derivative $[ArCH_2]^+$ at *m/z* 151 in the EIMS implicated the occurrence of a 3,4-dimethoxyphenyl substituent. The relative stereochemistry of H-7 and H-8 of **112** was determined as *trans*-oriented based on the observed coupling constant value ($J_{7,8}$ = 8.8 Hz), which is larger than when these protons are *cis*-oriented ($J$ = 6.3 Hz), as in sylvone [61]. Furthermore, the *cis*-configuration of H-8 and H-8' was confirmed by their strong NOESY interactions [51]. The $^1H$- and $^{13}C$-NMR signals were assigned by $^1H$-$^1H$ COSY, distortionless enhancement by polarization transfer (DEPT), heteronuclear correlated spectroscopy (HETCOR), and comparison with those of sylvone [61].

(–)-6'-Hydroxyyatein (**113**), a dibenzylbutyrolactone-type lignan, was obtained as a colorless amorphous powder [51]. Its molecular formula was established as $C_{22}H_{24}O_8$ by HREIMS at *m/z* 416.1469. The UV absorptions at 206, 230 sh, and 303 nm suggested the presence of a dibenzylbutyrolactone lignan skeleton [46]. The IR spectrum indicated that **113** contained a phenolic hydroxyl (3340 $cm^{-1}$), a lactone carbonyl (1758 $cm^{-1}$), and a methylenedioxy group (1030 and 930 $cm^{-1}$) [51]. The $^1H$ NMR spectrum of **113** exhibited eight aliphatic protons, three methoxyl groups, a methylenedioxy group, and four aromatic protons. The presence of 3,4,5-trimethoxybenzyl and 2-hydroxy-4,5-methylenedioxybenzyl moieties in **113** was supported by the observed prominent fragments at *m/z* 181 (76%) and 151 (100%), respectively, in its EIMS [51]. Its structure was confirmed by $^1H$-$^1H$ COSY and NOESY experiments [Fig. (**8**)]. The negative specific rotation value of **113** revealed the *R*-configurations at C-8 and C-8', similar to those of (–)-yatein (**123**) [46].

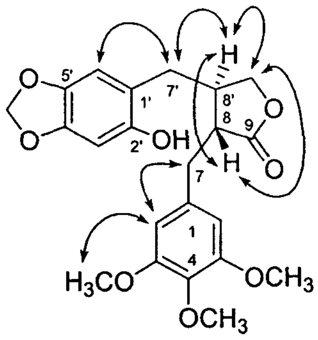



**Fig. (8).** Selected NOESY correlations observed for **113**.

## CONSTITUENTS OF *HERNANDIA* SPECIES WITH REPORTED BIOLOGICAL ACTIVITY

Biological evaluations of isolates from *Hernandia* species published to date have focused mainly on their cytotoxic, potential cancer chemopreventive, antiplatelet aggregation, and antimalarial activities. Bioactive compounds from the genus *Hernandia* with their individual activities of these types will be summarized specifically in the following paragraphs.

### Cytotoxicity against Cancer Cell Lines

In work carried out by Ih-Sheng Chen's group at Kaohsiung Medical College (Table 3), several isoquinoline and dimeric isoquinoline alkaloids as well as lignan constituents have been found to exhibit potent cytotoxicity against one or more cancer cell lines in a small tumor panel. Included were the KB-16 (human oral epidermal carcinoma), A549 (human lung adenocarcinoma), HT-29 (human colon carcinoma), and P-388 (murine lymphocytic leukemia) cell lines, with significant activity designated by $IC_{50}$ values of <4 μg/mL. Two lignans, (–)-deoxypodophyllotoxin (**101**) and (–)-yatein (**123**), showed highly potent activity against all of these four cell lines ($IC_{50}$ <0.005 μg/mL). Interestingly, other constituents of *Hernandia* species exhibited various degrees of selectivity towards the above-mentioned cell lines (Table 3). Despite the promising *in vitro* cytotoxicities listed in Table 3, none of

these compounds when isolated from *Hernandia* species appears to have been subjected to follow up *in vivo* evaluation of anti-tumor activity in a mouse model or human tumor xenograft system [62,63].

**Table 3. Cytotoxic Effects of Isolates from *Hernandia* Species Against Selected Human and Murine Cancer Cell Lines[a,b]**

| Compound type/name | Cell line [$IC_{50}$ (μg/mL)] | | | | Reference |
|---|---|---|---|---|---|
| | KB 16 | A549 | HT-29 | P-388 | |
| **ISOQUINOLINE ALKALOIDS** | | | | | |
| (+)-Corytuberine (**5**) | 1.00 | 1.86 | 5.75 | 0.35 | [30] |
| Demethylsonodione (**7**) | 0.51 | 0.22 | 0.77 | 0.77 | [30] |
| 7-Formyldehydrohernangerine (**9**) | 0.66 | 2.28 | 2.30 | 0.88 | [30] |
| 7-Formyldehydronornantenine (**10**) | 0.57 | 4.21 | 3.42 | 1.54 | [30] |
| 7-Formyldehydroovigerine (**11**) | 0.47 | 2.73 | 3.65 | 1.75 | [30] |
| (+)-*N*-Formyldehydroovigerine (**12**) | 0.07 | 0.39 | 0.51 | 0.30 | [5] |
| (+)-*N*-Formylnornantenine (**14**) | >50 | >50 | 0.49 | 1.91 | [30] |
| (+)-*N*-Formylovigerine (**15**) | 1.56 | 1.75 | 0.58 | 1.62 | [30] |
| Hernandonine (**18**) | 0.63 | 0.09 | 0.35 | <0.001 | [30] |
| Hernanymphine (**20**) | 0.64 | 0.68 | 0.47 | 0.54 | [30] |
| (+)-Hernovine (**21**) | 12.89 | 14.31 | 5.99 | 0.21 | [5] |
| 7-Hydroxy-6-methoxy-1-methylisoquinoline (**22**) | 0.22 | 2.21 | 1.94 | 0.80 | [30] |
| (+)-*N*-Hydroxyhernangerine (**23**) | 0.62 | 6.26 | 1.62 | 2.05 | [5] |
| *N*-Hydroxyovigerine (**24**) | 0.09 | 0.11 | 1.03 | 0.13 | [30] |
| (+)-Laurotetanine (**30**) | 6.25 | 3.36 | 1.82 | 0.15 | [5] |
| (+)-Magnoflorine (**32**) | >50 | 13.56 | 13.50 | 0.23 | [5] |
| *N*-Methylcorydaldine (**35**) | 0.99 | 2.14 | 0.93 | 0.22 | [30] |

**Table 3. Cytotoxic Effects of Isolates from *Hernandia* Species Against Selected Human and Murine Cancer Cell Lines (Continued)**

| Compound type/name | Cell line [$IC_{50}$ (μg/mL)] | | | | Reference |
|---|---|---|---|---|---|
| | KB-16 | A549 | HT-29 | P-388 | |
| (+)-*N*-Methylhernangerine (**37**) | 1.95 | >50 | 0.79 | 0.08 | [30] |
| *N*-Methylovigerine (**41**) | 0.33 | 0.47 | 0.90 | 0.19 | [30] |
| (+)-Ovigerine (**50**) | 0.53 | 0.27 | 0.76 | 0.29 | [30] |
| 7-Oxohernagine (**52**) | 0.33 | >50 | >50 | 1.87 | [30] |
| Thalifoline (**59**) | 0.89 | 1.48 | 1.75 | >50 | [5] |
| ISOQUINOLINE ALKALOID DIMERS | | | | | |
| (+)-Malekulatine (**70**) | 0.19 | 1.90 | 2.02 | 0.98 | [30] |
| (+)-Ovihernangerine (**73**) | 0.24 | 1.57 | 10.23 | 1.00 | [30] |
| (+)-Vateamine-2'β-*N*-oxide (**81**) | 0.53 | 2.15 | 1.87 | 0.39 | [30] |
| LIGNANS | | | | | |
| (–)-Deoxypodophyllotoxin (**101**) | <0.001 | <0.001 | <0.001 | <0.001 | [30] |
| (+)-Epiaschantin (**105**) | 0.70 | 2.78 | 1.75 | 1.45 | [30] |
| (+)-Epimagnolin (**107**) | 0.47 | 0.84 | 1.65 | 0.39 | [30] |
| (+)-Epiyangambin (**109**) | 0.26 | 2.27 | 2.33 | 0.39 | [30] |
| (–)-Hernone (**112**) | 1.22 | 1.12 | 0.91 | 0.81 | [51] |
| (–)-Nymphone (**119**) | 0.64 | 3.02 | 0.74 | 0.32 | [51] |
| (–)-Yatein (**123**) | <0.001 | <0.004 | <0.005 | <0.001 | [30] |

[a]Cytotoxicity is denoted by inhibition of human epidermal carcinoma (KB-16), human lung adenocarcinoma (A549), human colon carcinoma (HT-29), and murine lymphocytic leukemia (P-388) cells at half maximal inhibitory concentration ($IC_{50}$) values of μg/mL. [b]For the cell lines tested, an $IC_{50}$ value of <4 μg/mL is regarded as active.

## Cancer Chemoprevention

Cancer chemoprevention is defined as the use of natural or synthetic agents to inhibit, retard, or reverse the process of carcinogenesis [64,65]. Various constituents of fruits and vegetables have been found to prevent cancer formation in full-term carcinogenesis inhibition studies in experimental animals [65]. Since 1987, in the cancer chemoprevention testing program of the United States National Cancer Institute (NCI), Bethesda, Maryland, more than 40 promising agents and agent combinations have been evaluated clinically as potential chemopreventive drugs for major cancer targets [66,67].

A program project at the University of Illinois at Chicago in the aim of cancer chemoprevention, entitled "Natural Inhibitors of Carcinogenesis" (P01 CA48112), has been supported by the NCI since 1991 [68-73]. This program project is organized with close collaboration of botanical, biological, chemical, biostatistical, analytical, and administrative components [69,72]. In brief, the plant materials for investigation are acquired by utilizing the resources of the University of Illinois Pharmacognosy Field Station and the herbarium of the Field Museum of Natural History, Chicago, as well as commercial sources and by field collection through interactions with international botanist collaborators. Priority in plant collections has been given to edible species [74]. Initially, a small amount (100-1000 g) of each plant material sample is collected for preliminary investigation. Crude non-polar and polar extracts are prepared from each plant obtained, which are then evaluated for their potential chemopreventive activity using a battery of about ten short-term *in vitro* bioassays relating to cancer chemoprevention [68-70]. Selected extracts are then evaluated in a mouse mammary organ culture model as a secondary discriminator [75]. In the next stage, plant extracts showing potency and/or selectivity in the *in vitro* bioassay models are selected for bioassay-guided fractionation to isolate the active principles using various modern chromatographic techniques. Pure active compounds are then evaluated in all of the *in vitro* assays, with selected compounds then progressing to the mouse mammary organ culture model. Meanwhile, the chemical structures of bioactive constituents are determined by spectroscopic analysis and chemical transformation methods [76-78]. The *in vivo* cancer chemopreventive activity of highly promising pure plant constituents is then evaluated in a two-stage mouse

skin and/or a rat mammary carcinogenesis model [79,80]. Agents judged to have potential as human chemopreventives will be subjected to preclinical toxicology and pharmacokinetic studies [81,82], and then hopefully to Phase I clinical safety and pharmacokinetic trials [83]. Finally, it is intended that the most successful agents will progress to clinical chemoprevention trials [84].

The development of carcinogenesis in a mammalian cell may be represented by three discrete but often overlapping stages, inclusive of the initiation, promotion, and progression phases [65]. 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) and other diterpene esters from the plant family Euphorbiaceae are well-known as tumor-promotion agents [85]. Thus, inhibition of the biological effects induced by TPA has been considered as a promising strategy [68]. To date, two short-term cell-based *in vitro* bioassays using TPA as a tumor-promoter have been developed to evaluate the potential cancer chemopreventive activities of several lignans isolated from *Hernandia* species, namely, inhibition of Epstein-Barr virus early antigen activation [6] and the inhibition of the transformation of JB6 mouse epidermal cells [7].

The first of these assay systems has been carried out by C. Ito and colleagues at Meijo University, Japan, and utilizes the Epstein-Barr virus (EBV) in Raji cells (EBV genome-carrying human lymphoblastoid cells; EBV non-producer type) as the indicator, *n*-butyrate as the EBV-inducer, TPA as the tumor promoter, and the test substance [6,86]. This bioassay was based on the finding that the expression of the latent EBV genome in Raji cells can be induced by tumor promoters and more markedly enhanced by the combination of *n*-butyrate and TPA [87]. Recently, seven lignans from *Hernandia ovigera*, inclusive of bursehernin (**94**), epimagnolin (**107**), 1,2,3,4-dehydrodeoxypodophyllotoxin (**97**), 1,2,3,4-dehydropodophyllotoxin (**98**), 6,7-demethylenedeoxypodophyllotoxin (**99**), deoxypodophyllotoxin (**101**), and podorhizol (**122**), were reported to exhibit significant inhibitory effects on EBV early antigen activation induced by TPA in Raji cells, and showed $IC_{50}$ values corresponding to 9.4, 11.8, 10.4, 9.4, 10.2, 11.0, and 9.6 µg/mL, respectively [6].

A second bioassay, a soft agar transformation assay, has been utilized at the University of Illinois at Chicago to determine the inhibition of transformation of the JB6 cell line by treatment of the test materials [88]. The primary epidermal cell cultures from newborn BALB/c mice have been previously well developed for use as a tool in chemical

carcinogenesis studies [89-91]. Of these cultures, the JB6 mouse epidermal cell line was found to respond to tumor-promoting but not to non-promoting phorbol esters with an irreversible induction of the tumor-cell phenotype, which could be measured by colony formation in 0.33% agar at 14 days [92]. Also, the JB6 precursor cells are non-tumorigenic while the phorbol ester-induced anchorage-independent transformants are tumorigenic [92]. Furthermore, it has been considered that the process of induction of anchorage independence by tumor-promoting phorbol esters in the JB6 cell line may mimic a late phase of tumor promotion *in vivo* in which 12-*O*-tetradecanoylphorbol 13-acetate (TPA) apparently transforms benign premalignant lesions to invasive tumors irreversibly [93]. Based on the above observations, a soft agar transformation assay has been utilized to determine the inhibition of transformation of the JB6 cell line by treatment of the test materials [88]. In order to ensure whether the inhibitory activity could be attributed to cytotoxicity rather than a specific anti-promotion or anti-progression activity, a cytotoxicity assay against JB6 cells was used concomitantly to evaluate the cytotoxic potential of test materials [88]. Activity-guided fractionation of an ethyl acetate extract of the twigs of *Hernandia ovigera*, using the soft agar transformation assay with JB6 mouse epidermal cells, led to the isolation of two active lignans, (7*R*,8*S*,7'*R*,8'*R*)-(+)-7'-acetylpicropodophyllin (**91**) and (+)-epiyangambin (**109**), with $IC_{50}$ values of 0.33 and 0.90 μM, respectively. Compounds **91** and **109** were further tested for their cytotoxicity against the host cells, and exhibited $IC_{50}$ values of 2.6 and 12.8 μM, respectively [7]. The threshold for significant JB6 cell transformation inhibitory activity was taken as $IC_{50}$ <50 μM, and 13-*cis*-retinoic acid, with an $IC_{50}$ value of 0.021 μM, was used as the positive control [88].

So far, no potential cancer chemopreventive agent constituent from a *Hernandia* species has been subjected to biological follow up after the initial *in vitro* investigation, e.g., by using a mouse mammary organ culture assay [75] or a two-stage mouse skin carcinogenesis test [86].

## Antiplatelet Aggregation Activity

It is well known that platelets play an important role in the hemostatic process, and their aggregation can lead to arterial thrombosis [94]. Therefore, evaluation of compounds with antiplatelet aggregation activity

may lead to the discovery of potential drugs for the treatment of cardiovascular diseases. When evaluated in an *in vitro* antiplatelet aggregation turbidimetry assay carried out by I.-S. Chen *et al.* at Kaohsiung Medical College [4,24], nearly twenty isoquinoline alkaloids and lignans, obtained from *Hernandia* species, have been found to inhibit platelet aggregation induced by arachidonic acid (AA), collagen, platelet-activating factor (PAF), and thrombin, as listed in Table 4. Based on the historical data observed for this assay, compounds are classified as active if the $IC_{50}$ value is less than 300 μM [24]. Aspirin, a cyclooxygenase inhibitor [95], was used a positive control substance, which completely inhibited AA-induced platelet aggregation at 50 μM, but not that of other inducers [4,94]. Interestingly, three furofuran lignans (**105**, **107**, and **109**) exhibited the strongest and most selective antiplatelet activity induced by PAF compared with other compounds tested, and showed $IC_{50}$ values of 2-5 μM.

**Table 4. Inhibitory Effects of Isolates From *Hernandia* Species on the Induced Aggregation of Washed Rabbit Platelets[a,b]**

| Compound type/name | $IC_{50}$ (μM) | | | | Reference |
|---|---|---|---|---|---|
| | AA (100 μM) | Collagen (10 μg/mL) | PAF (2 nM) | Thrombin (0.1 U/mL) | |
| **BENZENOID** | | | | | |
| Isovanillin (**86**) | <150 | >150 | >150 | >150 | [24] |
| **ISOQUINOLINE ALKALOIDS** | | | | | |
| Dehydrohernandaline (**6**) | >100 | >100 | <100 | >100 | [4] |
| (+)-Hernandaline (**17**) | <50 | <50 | <50 | >50 | [4] |
| Hernangerine (**19**) | <300 | <300 | >300 | >300 | [24] |
| (+)-Laurotetanine (**30**) | 20-50 | <100 | 50-100 | >100 | [4] |
| (+)-Malekulatine (**70**) | 50-100 | <300 | 50-100 | >300 | [24] |
| *N*-Methylhernangerine (**37**) | <100 | <300 | >300 | >300 | [24] |
| (+)-Ovigerine (**50**) | <150 | <300 | 150-300 | >300 | [24] |

**Table 4. Inhibitory Effects of Isolates From *Hernandia* Species on the Induced Aggregation of Washed Rabbit Platelets (Continued)**

| Compound type/name | IC₅₀ (μM) | | | | Reference |
|---|---|---|---|---|---|
| | AA (100 μM) | Collagen (10 μg/mL) | PAF (2 nM) | Thrombin (0.1 U/mL) | |
| 7-Oxohernagine (**52**) | 20-50 | <100 | 20-50 | >100 | [4] |
| (+)-Reticuline (**57**) | 20-50 | 50-100 | >100 | >100 | [4] |
| (+)-Thalicarpine (**76**) | 50-100 | 20-50 | 10-20 | >100 | [4] |
| (+)-Vateamine-2'β-*N*-oxide (**81**) | 20-50 | 10-20 | 20-50 | >100 | [4] |
| LIGNANS | | | | | |
| (−)-Deoxypodophyllotoxin (**101**) | 50-100 | 50-100 | <100 | >100 | [4] |
| (+)-Epiaschantin (**105**) | >100 | >100 | 2-5 | >100 | [4] |
| (+)-Epimagnolin (**107**) | 50-100 | >100 | 2-5 | >100 | [4] |
| (+)-Epiyangambin (**109**) | >100 | >100 | 2-5 | >100 | [4] |
| (−)-Hernone (**112**) | >100 | >100 | 50-100 | >100 | [4] |
| (−)-Yatein (**123**) | 50-100 | 20-50 | 20-50 | >100 | [4] |

[a]Platelets were preincubated with DMSO (0.5%, control) or each compound at 37 °C for 3 minutes, and the inducer [arachidonic acid (AA), collagen, platelet-activating factor (PAF), or thrombin] was added.
[b]Activity denoted by antiplatelet aggregation at $IC_{50}$ (half maximal inhibitory concentration) values of <300 μM.

## Antimalarial Activity

A useful strategy in attempts to improve the efficacy of antimalarial drugs is the search for compounds, known as resistance-modifiers or chemosensitizers, that may restore chloroquine sensitivity in resistant stains of *Plasmodium* species [12,18]. In work performed by Ratsimamanga-Urverg *et al.* at Institut Malgache de Recherches Appliquées (IMRA), Antananarivo, Madagascar, four isoquinoline dimers, hervelines A-D (**66-69**), along with two isoquinolines, laudanosine (**29**) and reticuline (**57**), were evaluated for their intrinsic and chloroquine-potentiating antimalarial activities [12,39]. Among these alkaloids,

hervelines A-D showed moderate intrinsic *in vitro* antiplasmodial activity in a dose-dependent manner ($IC_{50}$ values in the range 1.69-3.28 μM), but displayed different modes of action in a chloroquine combination evaluation, with a synergistic effect by hervelines B and C, a simple additive effect by herveline A, and antagonism by herveline D. Hervelines A-C (**66-68**) were also evaluated for their cytotoxicity against uterine carcinoma (HeLa) cells and exhibited $IC_{50}$ values of >45.0, 14.2, and >45.0 μM, respectively. Furthermore, the antiplasmodial activity of chloroquine was potentiated *in vitro* by laudanosine (**29**) and reticuline (**57**) rather than herveline D (**69**).

Moreover, an *in vivo* antimalarial assay has been established to evaluate the *in vivo* intrinsic activity and chloroquine-enhancing effectiveness of alkaloids [96,97]. In brief, an aqueous solution of the test alkaloid (as citrates) was prepared so that 0.4 mL of solution corresponded to a dose of 10 mg/kg. Chloroquine was dissolved in 0.9% NaCl to obtain the concentrations of 0.75 and 1.50 mg/kg in a 0.2 mL aliquot. Swiss mice (6-8 weeks, 20±2 g) were randomized into four groups of 5-6 and inoculated intravenously in the tail vein on day 1 with $10^7$ parasitized erythrocytes obtained by suitable dilution with 0.9% NaCl of infected red blood cells from a donor mouse with rising parasitemia (20%) induced by a line of *P. yoelii* N67 resistant to chloroquine. The first group of mice was treated once daily starting on day 1 with 0.4 mL of test compound by the oral route. The second group received daily 0.2 mL of chloroquine administered subcutaneously. The third group was treated once a day simultaneously with 0.4 mL of test compound by gavage and 0.2 mL of chloroquine by subcutaneous injection. A group of untreated controls received the vehicle only. Treatments were repeated for an additional three days. On the fifth day, the percentage of parasitized red blood cells was determined using microscope slides containing a blood smear obtained from the tail and stained with Diff Quick reagent. The percentage of parasitemia of each mouse in all the groups was recorded and the mean percentage of each group was calculated by a common statistical procedure (Student's *t*-test). Deaths occurring during the test procedure were considered to be due to the toxicity of the test materials. Three separate experiments were made. The results showed that hervelines A (**66**) and B (**67**) exhibited *in vivo* antiplasmodial activities at a dose of 10 mg/kg although their *in vivo* activity profiles were different from the *in vitro* studies [12]. Compounds **66** and **67**

inhibited parasitemia by 31.8% and 15.1% *in vivo* while they exhibited $IC_{50}$ values of 3.28 and 1.68 μM *in vitro*, respectively. However, the synergistic effect between chloroquine (0.75 mg/kg) and herveline B, along with the simple additive effect between chloroquine (0.75 mg/kg) and herveline A, were confirmed in the murine model *in vivo*. In addition, the chloroquine-potentiating antiplasmodial activity of laudanosine (**29**) observed *in vitro* in drug combination was also confirmed by the *in vivo* studies [12]. Importantly, the excellent antiplasmodial effects of these isoquinoline alkaloids were shown to correlate with the ethnobotanical use of *H. voyronii* as an antimalarial and adjuvant to chloroquine [11,12].

## CONCLUSIONS

Three species of the genus *Hernandia*, *H. moerenhoutiana*, *H. nymphaeifolia*, and *H. voyronii*, have been of considerable interest for their ethnobotanical uses for the treatment of various human diseases, such as malaria, postpartum hemorrhage, retained placenta, and morning sickness. Extracts of *Hernandia* species have exhibited various biological activities including antioxidant, antimalarial, cytotoxic, and spasmodic activities, along with inhibitory activities of platelet aggregation and prostaglandin biosynthesis.

Phytochemical studies on *Hernandia* species have resulted in the isolation of over 130 major secondary metabolites, which represent six compound classes and comprise 83 alkaloids, three benzenoids, three flavonoids, 34 lignans, one monoterpenoid, and eight sterols. The alkaloidal constituents can be further divided into two major types represented by 59 isoquinolines and 22 isoquinoline dimers. The structures of these purified isoquinoline alkaloids and lignans from *Hernandia* species were determined mainly by a combination of the analysis of NMR data including $^1H$-$^1H$ COSY, HMQC, HMBC, and NOESY (ROESY) 2D-NMR techniques and fragmentation patterns of diagnostic ions in electron impact mass spectra, especially for certain isoquinoline dimers. About 30 isoquinoline alkaloids and 13 lignans were found to exhibit at least one biological activity, such as cytotoxicity against cancer cell lines, *in vitro* activity germane to cancer chemoprevention, antiplatelet aggregation, or an antiplasmodial effect. Furthermore, three isoquinoline alkaloids, herveline A (**66**), herveline B (**67**), and laudanosine (**29**), have shown *in vivo* antimalarial effects

Therefore, secondary metabolites such as isoquinoline alkaloids and lignans, from the remaining unstudied species in the genus *Hernandia*, seem worthy of more extensive chemical and biological investigation.

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## REFERENCES

[1] Verdcourt, B. In *Flora of Tropical East Africa*; R.M. Polhill, Ed.; A.A. Balkema Publishers: Rotterdam, **1985**, pp. 1-11.

[2] Kubitzki, K. In *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*; A. Engler, F.R. Markgraf, and T.H. Eckardt, Eds.; E. Schweizerbart'sche Verlagsbuchhandlung Science Publishers: Stuttgart, **1969**, Vol. *89*, pp. 78-209.

[3] Heywood, V.H.; Moore, D.M.; Stearn, W.T. *Flowering Plants of the World (Updated Edition)*. Oxford University Press: New York, **1993**.

[4] Chen, J.J.; Chang, Y.L.; Teng, C.M.; Chen, I.S. *Planta Med.* **2000**, *66*, 251-256.

[5] Chen, I.S.; Chen, J.J.; Duh, C.Y.; Tsai, I.L.; Chang, C.T. *Planta Med.*, **1997**, *63*, 154-157.

[6] Ito, C.; Itoigawa, M.; Ogata, M.; Mou, X.Y.; Tokuda, H.; Nishino, H.; Furukawa, H. *Planta Med.*, **2001**, *67*, 166-168.

[7] Gu, J.-Q.; Park, E.J.; Totura, S.; Riswan, S.; Fong, H.H.S.; Pezzuto, J.M.; Kinghorn, A.D. *J. Nat. Prod.*, **2002**, *65*, 1065-1068.

[8] Arimoto, M.; Yamaguchi, H.; Nishibe, S. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science B.V.: Amsterdam, **1996**, Vol. *18*, pp. 551-660.

[9] Cronquist, A. *The Evolution and Classification of Flowing Plants (Second Edition)*. New York Botanical Garden: Bronx, New York, **1988**.

[10] Walters, D.R.; Keil, D.J. *Vascular Plant Taxonomy (Fourth Edition)*. Kendall/Hunt Publishing Company: Dubuque, Iowa, **1996**.

[11] Rasoanaivo, P.; Ratsimamanga-Urverg, S.; Galeffi, C.; Nicoletti, M.; Frappier, F.; Martin, M.T. *Tetrahedron*, **1995**, *51*, 1221-1228.

[12] Rasoanaivo, P.; Ratsimamanga-Urverg, S.; Rafatro, H.; Ramanitrahasimbola, D.; Palazzino, G.; Galeffi, C.; Nicoletti, M. *Planta Med.*, **1998**, *64*, 58-62.

[13] Singh, Y.N.; Ikahihifo, T.; Panuve, M.; Slatter, C. *J. Ethnopharmacol.*, **1984**, *12*, 305-329.

[14] Ostraff, M.; Anitoni, K.; Nicholson, A.; Booth, G.M. *J. Ethnopharmacol.*, **2000**, *71*, 201-209.
[15] Dunstan, C.A.; Noreen, Y.; Serrano, G.; Cox, P.A.; Perera, P.; Bohlin, L. *J. Ethnopharmacol.*, **1997**, *57*, 35-56.
[16] Cox, P.A.; Sperry, L.R.; Tuominen, M.; Bohlin, L. *Econ. Bot.*, **1989**, *43*, 487-497.
[17] Masuda, T.; Yonemori, S.; Oyama, Y.; Takeda, Y.; Tanaka, T.; Andoh, T.; Shinohara, A.; Nakata, M. *J. Agric. Food Chem.*, **1999**, *47*, 1749-1754.
[18] Ratsimamanga-Urverg, S.; Rasoanaivo, P.; Milijaona, R.; Rakotoarimanga, J.; Rafatro, H.; Robijaona, B.; Rakoto-Ratsimamanga, A.; Verdier, F.; Bras, J.L. *Phytother. Res.*, **1994**, *8*, 18-21.
[19] Chen, J.J.; Tsai, I.L.; Ishikawa, T.; Wang, C.J.; Chen, I.S. *Phytochemistry*, **1996**, *42*, 1479-1484.
[20] Lavault, M.; Debray, M.M.; Bruneton, J. *Bull. Mus. Natl. Hist. Nat., Sect. B*, **1980**, *2*, 387-389.
[21] Richomme, P.; Lavault, M.; Jacquemin, H.; Bruneton, J. *Planta Med.*, **1984**, *50*, 20-22.
[22] Chalandre, M.C.; Pareyre, C.; Bruneton, J. *Ann. Pharm. Fr.*, **1984**, *42*, 317-321.
[23] Chen, I.S.; Chen, J.J.; Tsai, I.L. *Phytochemistry*, **1995**, *40*, 983-986.
[24] Chen, I.S.; Chen, J.J.; Tsai, I.L.; Chang, Y.L.; Teng, C.M. *Planta Med.*, **1995**, *61*, 537-539.
[25] Krane, B.D.; Shamma, M. *J. Nat. Prod.*, **1982**, *45*, 377-384.
[26] Chen, I.S.; Chen, J.J.; Tsai, I.L. *Heterocycles*, **1996**, *43*, 799-807.
[27] Bruneton, J. *C. R. Seances Acad. Sci., Ser. C*, **1980**, *291*, 187-189.
[28] Lavault, M.; Debray, M.M.; Bruneton, J. *Planta Med.*, **1981**, *42*, 50-54.
[29] Yakushijin, K.; Sugiyama, S.; Mori, Y.; Murata, H.; Furukawa, H. *Phytochemistry*, **1980**, *19*, 161-162.
[30] Chen, J.J.; Ishikawa, T.; Duh, C.Y.; Tsai, I.L.; Chen, I.S. *Planta Med.*, **1996**, *62*, 528-533.
[31] Chen, J.J.; Tsai, I.L.; Chen, I.S. *J. Nat. Prod.*, **1996**, *59*, 156-158.
[32] Ito, K.; Furukawa, H. *Tetrahedron Lett.*, **1970**, *34*, 3023-3024.
[33] Yang, T.H.; Liu, S.C.; Lin, T.S.; Yang, L.M. *J. Chin. Chem. Soc.*, **1976**, *23*, 29-34.
[34] Yang, T.H.; Liu, S.C.; Lin, T.S. *J. Chin. Chem. Soc.*, **1977**, *24*, 91-92.
[35] Chalandre, M.C.; Bruneton, J.; Cabalion, P.; Guinaudeau, H. *Can. J. Chem.*, **1986**, *64*, 123-126.
[36] Sultankhodzhaev, M.N.; Beshitashvili, L.V.; Yunusov, M.S.; Yagudov, M.R.; Yunusov, S.Y. *Khim. Prir. Soedin.*, **1980**, 665-672.
[37] Bruneton, J.; Shamma, M.; Minard, R.D.; Freyer, A.J.; Guinaudeau, H. *J. Org. Chem.*, **1983**, *48*, 3957-3960.
[38] Chalandre, M.C.; Guinaudeau, H.; Bruneton, J. *C. R. Acad. Sci., Ser. 2*, **1985**, *301*, 1185-1187.
[39] Ratsimamanga-Urverg, S.; Rasoanaivo, P.; Rafatro, H.; Robijaona, B.; Rakoto-Ratsimamanga, A. *Ann. Trop. Med. Parasitol.*, **1994**, *88*, 271-277.
[40] Ito, C.; Matsui, T.; Wu, T.S.; Furukawa, H. *Chem. Pharm. Bull.*, **1992**, *40*, 1318-1321.

[41] Yakushijin, K.; Shibayama, K.; Murata, H.; Furukawa, H. *Heterocycles*, **1980**, *14*, 397-402.
[42] Richomme, P.; Bruneton, J.; Cavé, A. *Heterocycles*, **1985**, *23*, 309-312.
[43] Richomme, P.; Bruneton, J.; Cabalion, P.; Debray, M.M. *J. Nat. Prod.*, **1984**, *47*, 879-881.
[44] Yamaguchi, H.; Arimoto, M.; Yamamoto, K.; Numata, A. *Yakugaku Zasshi*, **1979**, *99*, 674-677.
[45] Yamaguchi, H.; Arimoto, M.; Tanoguchi, M.; Numata, A. *Yakugaku Zasshi*, **1981**, *101*, 485-488.
[46] Tanoguchi, M.; Arimoto, M.; Saika, H.; Yamaguchi, H. *Chem. Pharm. Bull.*, **1987**, *35*, 4162-4168.
[47] Tanoguchi, M.; Hosono, E.; Kitaoka, M.; Arimoto, M.; Yamaguchi, H. *Chem. Pharm. Bull.*, **1991**, *39*, 1873-1876.
[48] Udino, L.; Abaul, J.; Bourgeois, P.; Gorrichon, L.; Duran, H.; Zedde, C. *Planta Med.*, **1999**, *65*, 279-281.
[49] Yamaguchi, H.; Arimoto, M.; Tanoguchi, M.; Ishida, T.; Inoue, M. *Chem. Pharm. Bull.*, **1982**, *30*, 3212-3218.
[50] Rahmani, M.; Toia, R.F.; Croft, K.D. *Planta Med.*, **1995**, *61*, 487-488.
[51] Chen, I.S.; Chen, J.J.; Duh, C.Y.; Tsai, I.L. *Phytochemistry*, **1997**, *45*, 991-996.
[52] Gottlieb, O.R.; Guimaraes, I.S.S.; Magalhaes, M.T.; Mesquita, A.A.L.; De Oliveira, W.G. *Acta Amazonica*, **1980**, *10*, 425-427.
[53] Kiryakov, H.G.; Iskrenova, E.S. *Planta Med.*, **1984**, *50*, 136-138.
[54] Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.*, **1988**, *51*, 389-474.
[55] Pharadai, K.; Pharadai, T.; Tantisewie, B.; Guinaudeau, H.; Freyer, A.J.; Shamma, M. *J. Nat. Prod.*, **1985**, *48*, 658-659.
[56] Guha, K.P.; Mukherjee, B.; Mukherjee, R. *J. Nat. Prod.*, **1979**, *42*, 1-84.
[57] Schiff, P.L. *J. Nat. Prod.*, **1983**, *46*, 1-43.
[58] Tomita, M.; Lu, S.T.; Chen, Y.Y. *Yakugaku Zasshi*, **1966**, *86*, 763-766.
[59] Ikeda, R.; Nagao, T.; Okabe, H.; Nakano, Y.; Matsunaga, H.; Katano, M.; Mori, M. *Chem. Pharm. Bull.*, **1998**, *46*, 871-874.
[60] Feliciano, A.S.; Corral, J.M.M.D.; Gordaliza, M.; Castro, M.A. *Phytochemistry*, **1990**, *29*, 1335-1338.
[61] Banerji, A.; Sarkar, M.; Ghosal, T.; Pal, S.C.; Shoolery, J.N. *Tetrahedron*, **1984**, *40*, 5047-5052.
[62] Rose, W.C.; Schurig, J.E.; Meeker, J.B. *Anticancer Res.*, **1988**, *8*, 355-368.
[63] Mi, Q.; Lantvit, D.; Reyes-Lim, E.; Chai, H.Y.; Zhao, W.; Lee, I.S.; Peraza-Sanchez, S.; Ngassapa, O.; Kardono, L.B.S.; Riswan, S.; Hollingshead, M.G.; Mayo, J.G.; Farnsworth, N.R.; Cordell, G.A.; Kinghorn, A.D.; Pezzuto, J.M. *J. Nat. Prod.*, **2002**, *65*, 842-850.
[64] Sporn, M.B.; Suh, N. *Carcinogenesis*, **2000**, *21*, 525-530.
[65] Surh, Y.J. *Mutat. Res.*, **1999**, *428*, 305-327.
[66] Kelloff, G.J.; Hawk, E.T.; Karp, J.E.; Crowell, J.A.; Boone, C.W.; Steele, V.E.; Lubet, R.A.; Sigman, C.C. *Sem. Oncol.*, **1997**, *24*, 241-252.
[67] Kelloff, G.J.; Sigman, C.C.; Greenwald, P. *Eur. J. Cancer*, **1999**, *35*, 1755-1762.

[68] Pezzuto, J.M.; Song, L.L.; Lee, S.K.; Shamon, L.A.; Mata-Greenwood, E.; Jang, M.; Jeong, H.-J.; Pisha, E.; Mehta, R.G.; Kinghorn, A.D. In *Chemistry, Biology and Pharmacological Properties of Medicinal Plants from the Americas*; K. Hostettmann, M.P. Gupta, and A. Marston, Eds.; Harwood Academic Publishers: Amsterdam, **1999**, pp. 81-110.

[69] Pezzuto, J.M. In *Human Medicinal Agents from Plants* (Symposium Series No. 534); A.D. Kinghorn and M.F. Balandrin, Eds.; American Chemical Society: Washington, DC, **1993**, pp. 204-215.

[70] Pezzuto, J.M. In *Recent Advances in Phytochemistry* (Volume 29): *Phytochemistry of Medicinal Plants*; J.T. Arnason, R. Mata, and J.T. Romeo, Eds.; Plenum Press: New York, **1995**, pp. 19-44.

[71] Kinghorn, A.D.; Fong, H.H.S.; Farnsworth, N.R.; Mehta, R.G.; Moon, R.C.; Moriarty, R.M.; Pezzuto, J.M. *Curr. Org. Chem.*, **1998**, *2*, 597-612.

[72] Pezzuto, J.M. Angerhofer, C.K.; Mehdi, H. In *Studies in Natural Product Chemistry* (Volume 20); Atta-ur-Rahman, Ed.; Elsevier Science: Amsterdam, **1998**, pp. 507-560.

[73] Kinghorn, A.D.; Su, B.N.; Lee, D.; Gu, J.Q.; Pezzuto, J.M. *Curr. Org. Chem.*, **2003**, *7*, 213-226.

[74] Fong, H.H.S.; Kosmeder II, J.W.; Farnsworth, N.R.; Soejarto, D.D.; Kinghorn, A.D.; Pezzuto, J.M. *Proceedings of the International Forum on Traditional Medicine, Toyama, Japan, October 16-17, 1999*, **2002**, pp. 63-72.

[75] Mehta, R.G.; Moon, R.C. *Anticancer Res.*, **1991**, *11*, 593-596.

[76] Gu, J.-Q.; Gills, J.J.; Park, E.J.; Mata-Greenwood, E.; Hawthorne, M.E.; Axelrod, F.; Chavez, P.I.; Fong, H.H.S.; Mehta, R.G.; Pezzuto, J.M.; Kinghorn, A.D. *J. Nat. Prod.*, **2002**, *65*, 532-536.

[77] Lee, D.; Bhat, K.P.L.; Fong, H.H.S.; Farnsworth, N.R.; Pezzuto, J.M.; Kinghorn, A.D. *J. Nat. Prod.*, **2001**, *64*, 1286-1293.

[78] Su, B.-N.; Cuendet, M.; Hawthorne, M.E.; Kardono, L.B.S.; Riswan, S.; Fong, H.H.S.; Mehta, R.G.; Pezzuto, J.M.; Kinghorn, A.D. *J. Nat. Prod.*, **2002**, *65*, 163-169.

[79] Jang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W.W.; Fong, H.H.S.; Farnsworth, N.R.; Kinghorn, A.D.; Mehta, R.G.; Moon, R.C.; Pezzuto J.M. *Science*, **1997**, *275*, 218-220.

[80] Udeani, G.O.; Gerhäuser, C.; Thomas, C.F.; Moon, R.C.; Kosmeder, J.W.; Kinghorn, A.D.; Moriarty, R.M.; Pezzuto, J.M. *Cancer Res.*, **1997**, *57*, 3424-3428.

[81] Steele, V.E.; Boone, C.W.; Lubet, R.A.; Crowell, J.A.; Holmes, C.A.; Sigman, C.C.; Kelloff, G.J. *Hematol. Oncol. Clin. North Am.*, **1998**, *12*, 943-961.

[82] Udeani, G.O.; Zhao, G.M.; Shin, Y.G.; Kosmeder, J.W.; Beecher, C.W.; Kinghorn, A.D.; Moriarty, R.M.; Moon, R.C.; Pezzuto, J.M. *Cancer Chemother. Pharmacol.*, **2001**, *47*, 263-268.

[83] Kelloff, G.J.; Johnson, J.R.; Crowell, J.A.; Boone, C.W.; DeGeorge, J.J.; Steele, V.E.; Mehta, M.U.; Temeck, J.W.; Schmidt, W.J.; Burke, G.; Greenwald, P.; Temple, R.J. *Cancer Epidemiol. Biomark. Prev.*, **1995**, *4*, 1-10.

[84] Kelloff, G.J.; Boone, C.W.; Crowell, J.A.; Steele, V.E.; Lubet, R.A.; Doody, L.A.; Malone, W.F.; Hawk, E.T.; Sigman, C.C. *J. Cell. Biochem.*, **1996**, *26* (Suppl.), 1-28.

[85] Hausen, H.; Bornkamm, G.W.; Schmid, R.; Hecker, H. *Proc. Natl. Acad. Sci. U.S.A.*, **1979**, *76*, 782-785.

[86] Ito, C.; Itoigawa, M.; Furukawa, H.; Ichiishi, E.; Mukainaka, T.; Okuda, M.; Ogata, M.; Tokuda, H.; Nishino, H. *Cancer Lett.*, **1999**, *142*, 49-54.

[87] Ito, Y.; Yanase, S.; Fujita, J.; Harayama, T.; Takashima, M.; Imanaka, H. *Cancer Lett.*, **1981**, *13*, 29-37.

[88] Chang, L.C.; Song, L.L.; Park, E.J.; Luyengi, L.; Lee, K.J.; Farnsworth, N.R.; Pezzuto, J.M.; Kinghorn, A.D. *J. Nat. Prod.*, **2000**, *63*, 1235-1238.

[89] Yuspa, S.H.; Morgan, D.L.; Levy, J.A. *J. Natl. Cancer Inst.*, **1973**, *50*, 1561-1570.

[90] Yuspa, S.H.; Lichti, U.; Ben, T.; Patterson, E.; Hennings, H.; Slaga, T.J.; Colburn, N.; Kelsey, W. *Nature (Lond.)*, **1976**, *262*, 402-404.

[91] Colburn, N.H.; Bruegge, W.F.; Bates, J.R.; Gray, R.H.; Rossen, J.D.; Kelsey, W.H.; Shimada, T. *Cancer Res.*, **1978**, *38*, 624-634.

[92] Colburn, N.H.; Ozanne, S.; Lichti, U.; Ben, T.; Yuspa, S.H.; Wendel, E.; Jardini, E.; Abruzzo, G. *Ann. N.Y. Acad. Sci.*, **1981**, *359*, 251-259.

[93] Colburn, N.H.; Former, B.F.; Nelson, K.A.; Yuspa, S.H. *Nature (Lond.)*, **1979**, *281*, 589-591.

[94] Chia, Y.C.; Chen, K.S.; Chang, Y.L.; Teng, C.M. Wu, Y.C. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 3295-3300.

[95] Vane, J.R. *Nature (New Biol.)*, **1971**, *231*, 232-235.

[96] Peters, W.; Portus, J.H.; Robinson, B.L. *Ann. Trop. Med. Parasitol.*, **1975**, *69*, 155-171.

[97] Rasoanaivo, P.; Ratsimamanga-Urverg, S.; Milijaona, R.; Rafatro, H.; Rakoto-Ratsimamanga, A.; Galeffi, C.; Nicoletti, M. *Planta Med.*, **1994**, *60*, 13-16.

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# A SURVEY ON THE *HYPERICUM* GENUS: SECONDARY METABOLITES AND BIOACTIVITY

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**ABSTRACT**: Plants of the genus *Hypericum* have been used as traditional remedies in several parts of the world: among the different species, throughout the history, particularly *H. perforatum* (St. John's Wort) has gained high reputation as a wound-healing and an anti-inflammatory drug. However, the current use of the plant is mainly consistent with an antidepressant. The high biological value of St. John's Wort has worldwide led to an increased interest for the study of the chemical and pharmacological properties of other related species. The genus has been found to be a prolific source of various molecules with unique structure. Chemical and biological features of plants belonging to the genus *Hypericum* are described in the present paper

## INTRODUCTION

The genus *Hypericum, (Clusiaceae* Lindley, syn. *Guttiferae* A.L. de Jussieu) includes a large number of species worldwide distributed [1]. Several of the botanical species belonging to the genus are used in folk medicine and among them *H. perforatum* L. (St. John's Wort) is especially known as a traditional remedy for the treatment of melancholia, abdominal and urogenital pain and ulcerated burns [2,3]. Over the last two decades, it has been demonstrated that St. John's Wort is effective as an antidepressant [4-6], an antiviral [7,3] and an antimicrobial [8].

The drug contains peculiar chemical constituents such as naphtodianthrones (hypericins), acylphloroglucinols (hyperforins), flavonol glycosides (quercetin derivatives) and biflavones [9] and all the metabolites as a whole seem to contribute to its pharmacological activity. Current use of St. John's Wort is mainly for the treatment of mild to moderate depression [10,3] and drug extracts for antidepressant applications have become increasingly popular.

During the recent years, several phytochemical studies on other species of *Hypericum* have also been performed, leading to the isolation of new molecules, some having biological activity.

## BOTANY

The genus *Hypericum* belongs to the botanical family of the *Clusiaceae* Lindley. The family was formerly known as the *Guttiferae* (= gum-bearing) A.L. de Jussieu, due to the presence of secretory canals or cavities, containing the clear to coloured exudates typical of the plant group [1],

Three subfamilies are recognized within the *Clusiaceae*: the *Kielmeyeroideae*, the *Clusioideae* and the *Hypericoideae*. The last includes the genus *Hypericum* and it is often classified as a separate family, the *Hypericaceae,* paraphyletic to the *Clusiaceae* [1].

The genus *Hypericum* is divided into more than 50 sections including more than 400 species [11], which are widespread in temperate regions. In Europe, 18 sections with 61 species have been identified within the genus [11-14] and they can be differentiated on the basis of their morphological characters such as plant phenotype, position of leaves and organization of flowers and, number of stamens and carpels. However, secretion structures represent the most important diagnostic parameter to characterize the genus [11,14]. Pellucid and black/red dots or canals might be differently present on the various organs of the plant, Fig. (**1**), and they are usually related with the content of essential oils and hypericin pigments, respectively [1].

*H. perforatum* L. is the most diffuse species, generally wild growing in uncultivated areas. It is a herbaceous perennial plant, highly polymorphic, especially regarding the size of the leaves [11].

Varieties of *H. perforatum* with “broad” (var. *perforatum*) and “narrow” (var. *angustifolium*) sized leaves have been in fact recognized, together with plant populations with intermediate (var. *microphyllum*) phenotypic characters.

Pellucid glands are present on their leaves, while black secretory structures are normally found on the flowers.

Three subspecies of *H. perforatum* are reported in the Italian Flora: *H. perforatum* ssp. *perforatum* with large sized leaves; *H. perforatum* ssp. *angustifolium* (DC) Gaudin and ssp. *veronense* (Schrank) with narrow sized leaves [13,14].

*H. perforatum* ssp. *veronense* shows typical longitudinal dark canals on the petals, in addition to the usual dark secretory glands. Translucent glands are particularly abundant on the leaves of *H. perforatum,* which assume a perforated-like appearance when observed in transverse light.

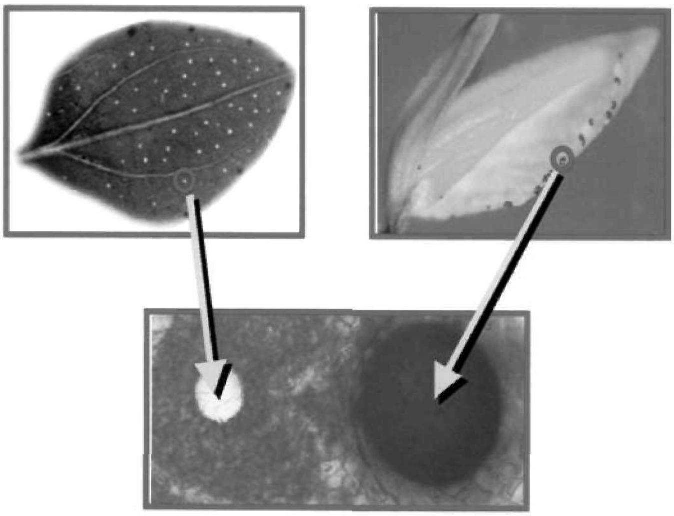


**Fig. (1).** Pellucid and black/red glands of *H. perforatum* (Kindly Prof. L. Maleci Bini, University of Florence, Italy)

# CHEMISTRY

## Essential Oils

Plants of hypericum typically have on their leaf surface translucid glands which contain essential oils. The first investigation on the chemistry of this class of metabolites, in the genus *Hypericum*, dates back to 1925 when α-pinene, some sesquiterpenes and an ester of isovaleric acid were indicated as possible constituents of the volatile oil from *H. perforatum*. About ten years later, *n*-nonane was identified in *Sarothra gentianoides*, a plant now classified as *Hypericum sarothra* [15]. Accumulation of the essential oils has been studied in detail in *H. perforatum* [9,16-22]. The highest content is reported for plants collected before flowering (0.26% *vs* 0.06-0.11% for flowering plants); moreover, plants without stems are reported to produce up to 0.35% of oils. Fresh flowers also represent a part of the plant accumulating high amounts (0.25%) of essential oils [22].

The essential oils from a number of *Hypericum* species proved to be useful chemotaxonomic markers for the genus [17-19]. Mathis and

Ourisson reported that the main constituents of hypericum essential oils are represented by hydrocarbons (methyl-2-octane and *n*-nonane), monoterpenes (limonene, α- and β-pinene), and sesquiterpenes (ß-caryophyllene). The relative content of limonene has been taken as a diacritic character to divide the genus into two groups: one including sections with a high content of limonene (> 10%), and the other comprehensive of plant sections containing less than 5% of limonene in their essential oils, Fig. (2) [17].

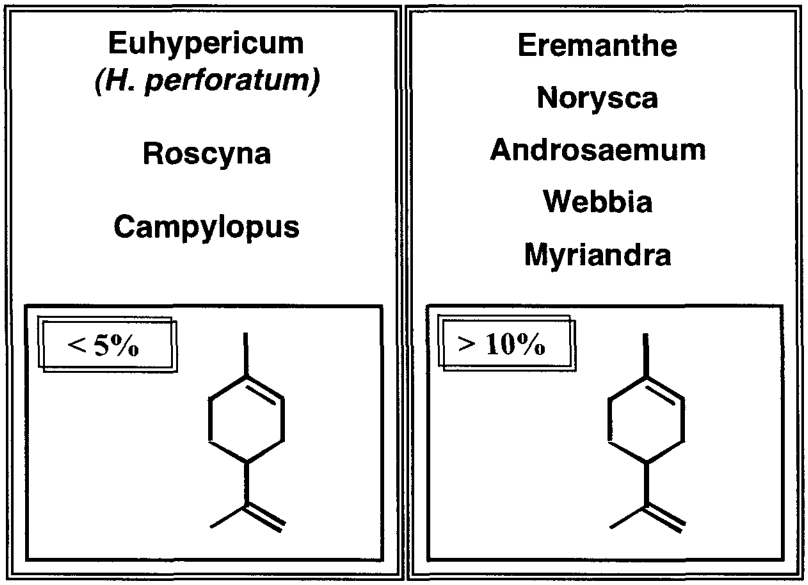



**Fig. (2).** Sections of *Hypericum* grouped according to the limonene content in their essential oils.

Furthermore, a detailed investigation of the volatiles from plant species belonging to the *Euhypericum* section has allowed to distinguish four groups based on the dominant constituents [17]. Thus, species have been characterized, such as *H. perforatum*, with high amounts of methyl-2-octane (≥ 30%); species containing in their essential oils linear hydrocarbons and traces of methyl-2-octane (for example, *H. hirsutum*, *H. tetrapterum* and *H. elegans*); species with α- and β-pinene as the main terpenes (*H. montanum*, *H. pulchrum* and *H. humifusum*); and finally

species producing terpenes and hydrocarbons, such as for example *H. barbatum*. However, according to the Authors, the last two groups of plants do not produce methyl-2-octane.

The above data have been generally confirmed by a later study concerning different species of hypericum native to Portugal [23]. More recent papers on the essential oils of *Hypericum* species have contributed to enlarge the number of identified monoterpenes and sesquiterpenes [23-29]. Thus, *H. perforatum* has been found to contain good amounts of ß-caryophyllene (2-13%), caryophyllene oxide (2-14%), germacrene D (6-47%) and spathulenol (6-9%). Recently, composition of the essential oils from *H. hircinum* has also been revised [29] and the following compounds have been found as the main components: nonane (35.5 and 19.3%, fruits and leaves, respectively), ß-pinene (17.3 and 0.3%), α-pinene (8.5 and 0.8%), limonene (12.7and 2.5%), α-gurjunene (10.7 and 0.3%) and caryophyllene oxide (15.3 and 0.3%)

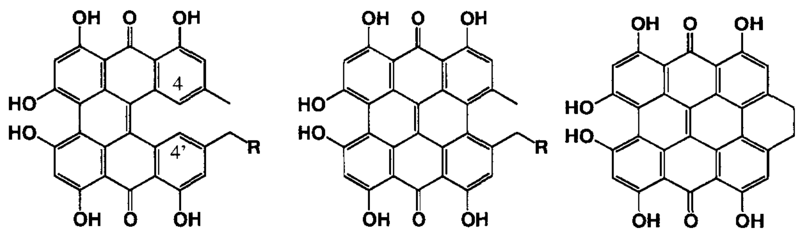
## Naphtodianthrones

Naphtodianthrones isolated from the genus *Hypericum* include hypericin ($C_{30}H_{16}O_8$, 10,11-dimethyl-1,3,4,6,8,13-hexahydroxynaphtodianthrone), pseudohypericin, Fig. (**3**), and isohypericin [2,3,9,10,22]. Those metabolites are powerful photosensitizers and they are believed to be responsible for hypericism, a photodermatosis which affects cattle grazing on *Hypericum* plants.

The so called protoderivatives, protohypericin and protopseudohypericin, lacking the 4,4'-bonds, have been isolated from fresh plant material and they represent the biosynthetic precursors of the more stable compounds, hypericin and pseudohypericin, respectively. Their conversion is catalyzed by light. Cyclopseudohypericin, an oxidation product derived from pseudohypericin, has also been isolated, Fig. (**3**).

Hypericin and related compounds are responsible for the red/dark colour of the glands found on the leaves and petals of *Hypericum* species. The pigment is not a common natural component: until now, it has been isolated only from *Hypericum* plants and from some species of insects [2,30], where it acts as a photoreceptor.

Isolation of hypericin, first named "hypericum red", dates back to the beginning of 1800; however, its correct structure and total synthesis was accomplished about one century later [10,31-33]. Hypericin, with inorganic



Protohypericin, R = H
Protopseudohypericin, R = OH

Hypericin, R = H
Pseudohypericin, R = OH

Cyclopseudohypericin

**Fig. (3).** Chemical structure of hypericin and related compounds.

bases, forms monobasic salts which are generally soluble in polar solvents. Free hypericin is instead slightly soluble in polar solvents, but highly soluble in pyridine and organic bases. The compound occurs in the plant mainly as potassium salt. Hypericin salts are red in organic solvents and show a typical absorbance at 590 nm, which is useful to quantify hypericin in the drug extracts [34].

The molecule exists as a tautomeric mixture, with the dominance of the 1,6-diketo tautomer in the solid state or in dry non-protic solvents. On addition of water or protic solvents, the 1,6-diketo tautomer isomerizes to the 7,14-diketo tautomer, which represents the stable monomeric form of hypericin [30].

Little is known about the biosynthetic origin of hypericin(s): they are related to the anthranoid metabolism and emodin anthrone is possibly their precursor. Synthesis *in vitro* of hypericin following alkaline dimerization of emodin and oxidation of its reduction derivative, emodine anthrone, has in fact been demonstrated [35,36].

Indirect evidence of the correlation between hypericin(s) formation and the anthranoid metabolism has been recently gained by *in vitro* experiments with cell cultures of *H. perforatum* [37]. Cell suspension cultures and calli were unable to produce the typical *in vivo* metabolites of the plant. Their extracts contained instead high amounts of xanthones. Hypericins were found only in plantlets regenerated from calli, which, on the contrary, did not produce xanthones. The lack of hypericin(s) production in cell suspensions and calli seems to be in contrast with their ability to synthesize xanthones, which arise by the same pathway leading to hypericin. The presence of xanthones in the immature cell cultures indicates in fact that, besides the shikimate pathway, the acetogeninic metabolic system is also active. On the other hand, the total absence of hypericins in undifferentiated cell cultures, where large amounts of xanthones are found, suggests that the enzymes involved in the anthranol dimerization to hypericins are lacking.

Until recently, quality of drug preparations based on hypericum has been standardized by the hypericin(s) content (0.3%), determined by spectrophotometric measurements [22,38]. For this reason, distribution of hypericin and related constituents in St. John's Wort has been extensively studied. It has been found that hypericin is present in all the aerial parts of the plant, particularly in its flowers and buds. Accumulation of the protohypericins is however higher in the fresh plant material [22,39-42].

A detailed investigation on the presence of hypericin in different species of *Hypericum* has been carried out by Mathis and Ourisson [43]. They have shown that the active compound is especially present in the *Euhypericum* (including *H. perforatum*) and *Campylosporus* sections. Nevertheless, hypericin is rare in the sections *Thasium*, *Norysca*, *Androsaemum* and *Humifusoideum,* Fig. (**4**). Those data have been partially confirmed by a later work of Kitanov [44].

The variation of the hypericin content has been analyzed in *H. perforatum* [22,40]. Flowers and leaves were the part of the plant accumulating higher quantities of hypericin (2150 *vs* 290-380 ppm). Plant varieties with "narrow leaves", with more glands compared to the "broad leaves" varieties ($6.2/mm^2$ *vs* $2.2/mm^2$) were found to produce larger amounts of hypericin (1040-1630 ppm *vs* 370-580 ppm).

Furthermore, it has been shown that hypericin/pseudohypericin content in the plant undergoes seasonal variations: in the broad leaf biotype of *H. perforatum*, the amount of hypericins varied from a winter minimum of less than 100 ppm to a summer maximum of about 3000 ppm. In contrast, the narrow leaf variety increased its content of hypericins from similar winter values to summer maxima of almost 5000 ppm [22,40,41].

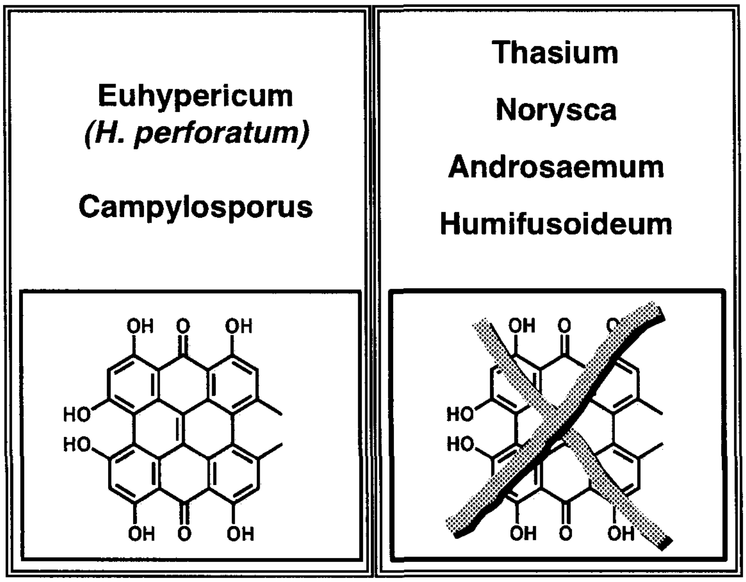



**Fig.** (4). Sections of *Hypericum* grouped according to their hypericin content.

## Phloroglucinols

Acylphloroglucinols are other characteristic constituents of the *Clusiaceae* plant family. They also occur in the *Cannabinaceae*, in the *Euphorbiaceae*, in the *Rosaceae* and in some ferns [9].

Hyperforin, $C_{35}H_{52}O_4$, 5-(2-methyl-1-oxo-propyl)-6-exo-methyl-1,3-endo,7-exo-tri-(3-methylbut-2-enyl)-6-endo-(4-methyl-pent-3-enyl)-byciclo [3.3.1]-nonan-2,4,9-trione, (2-4.5%) and adhyperforin (0.2-1.9%) are the main prenylated phloroglucinols isolated from *H. perforatum*, Fig. (**5**).

They are most abundant in the fruits and flowers from the fresh drug [3,9,22] and their relative amount is related to the plant stage of growth, being generally higher when the fruits are mature. As a consequence, quantification of hyperforin(s) is strictly dependent on the amount of flowers and fruits in the crude drug and plant harvesting at the beginning of fruit ripening would be preferable for their extraction.

The chemical structure of hyperforin has been deduced by spectroscopic means, as well as by chemical degradation and derivatization [45,46]. This lipophilic component of St. John's Wort consists of a mixture of photo- and oxygen-sensitive tautomers. Hyperforin is also unstable toward high temperature, on prolonged storage and in solution [9]. In particular, it has been shown that the compound is stable in protic solvents and unstable in apolar solvents such as *n*-hexane. It degrades at 40°C in presence of air [47,48].

An unusual related molecule, named hydroperoxycadiforin, Fig. (**6**), with the combined structure of a cadinan sesquiterpene and hyperforin, containing a hydroperoxide moiety, has been isolated from the stems and leaves of *H. perforatum* [49].

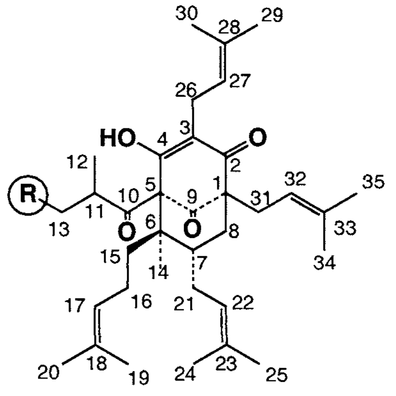
In the recent years, a number of analogues more polar than hyperforin have been characterized in *H. perforatum* [50-52]. Due to the limited stability of hyperforin(s), the question rises however, whether those compounds are genuine metabolites or artifacts produced during the isolation and purification procedures. Figure (7) illustrates some of the oxidative degradation products of hyperforin which have been isolated from St. John's Wort.

Recently [48], identification of degradation products of hyperforin has been accomplished by HPLC-ESI and $MS^n$ analyses: based on this study, furohyperforin (=orthoforin) and furohyperforin hydroperoxide should be considered as artifacts.

2-Methyl-3-buten-2-ol, a component of St. John's Wort essential oil, is also reported as a degradative product possibly obtained by oxidative cleavage of the isoprenyl side chain of hyperforin and adyhyperforin [9].

Phytochemical studies of different species of *Hypericum* indicated that the genus is a valuable source of secondary metabolites, with peculiar chemical features. Several other prenylated phloroglucinols, not related to hyperforin and sometimes unique in their structure, have been identified in *H. drummondii* [53], *H. brasiliense* [54], and *H. japonicum* [55].

As shown in Fig. (**8**), the basic structure of these constituents involves a phloroglucinol moiety (B-ring) and an acyl-filicinic acid moiety (A-ring), linked *via* a methylene bridge. Substitutions on the B-ring are characteristic: sarothralin *(1)* has an unsubstituted benzoyl group ($R_1$) and an isopentenyloxy group ($R_2$); sarothralen A *(2)* shows, instead, a geranyl side chain ($R_3$); sarothralin G *(3)* brings both, an unsubstituted benzoyl and a geranyl group on the B-ring.




R = H, Hyperforin

R = $CH_3$, Adhyperforin

**Fig. (5).** Chemical structure of hyperforins.

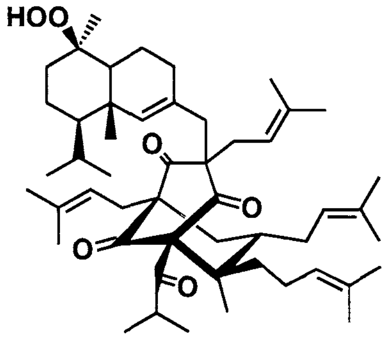



**Fig. (6).** Chemical structure of hydroperoxycadiforin.

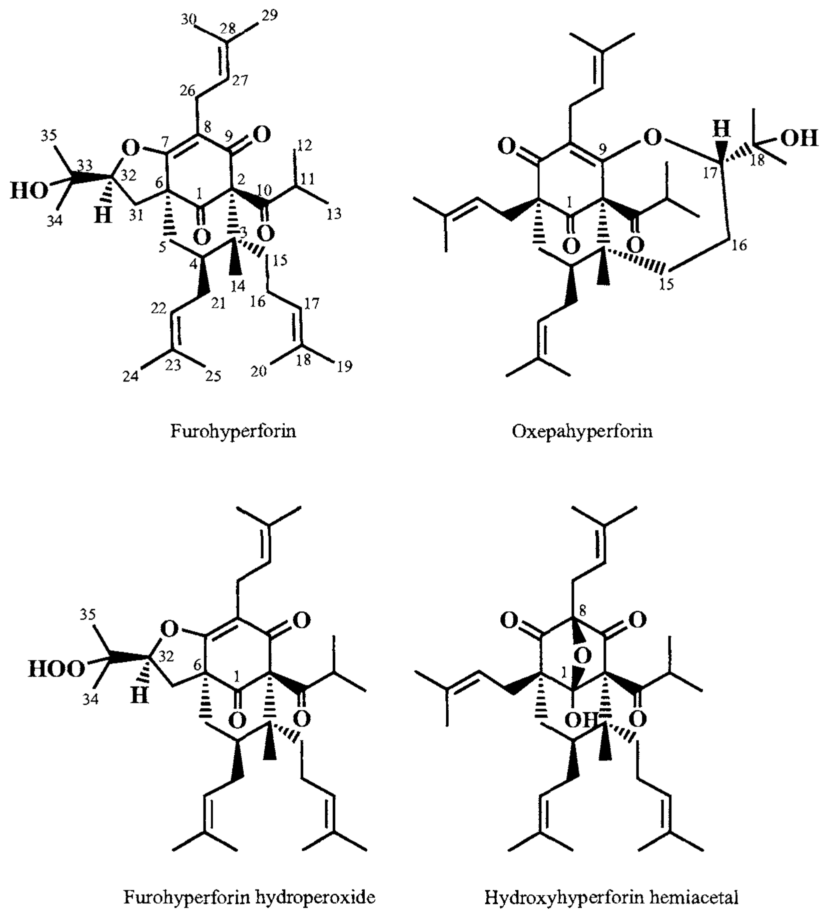



**Fig. (7).** Chemical structure of hyperforin oxidative degradation products.

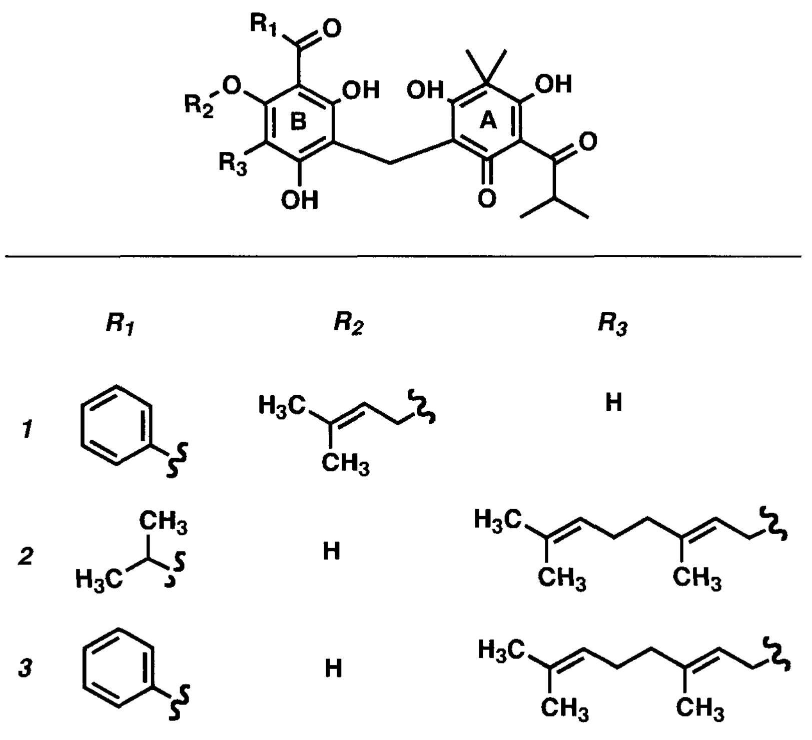



**Fig. (8).** Examples of prenylated phloroglucinols from *H. japonicum*.

It is also noteworthy that some of the phloroglucinols isolated from *Hypericum* species, are characterized by *O*-prenylation, which, in contrast to *C*-prenylation, is quite rare for natural phenolic compounds.

*H. japonicum,* a Chinese herbal drug used in the treatment of bacterial diseases, hepatitis and gastrointestinal disorders showed to be a rich source of unusual secondary metabolites [55-58]. In addition to the above described products, another *O*-prenylated phloroglucinol, the 2-acetyl-3,5-dihydroxy-1-geranoxy-6-methyl-4-(2-methyl) butyryl-benzene, bearing the rare geranoxyl group, has been identified [57] in this plant, Fig. (**9**). Prenylation with a geranyl chain seems however peculiar of the genus *Hypericum*: another example is represented by the diterpenol, 9-geranyl-α-terpineol or prenyl-α-bisabolol, Fig (**10**).

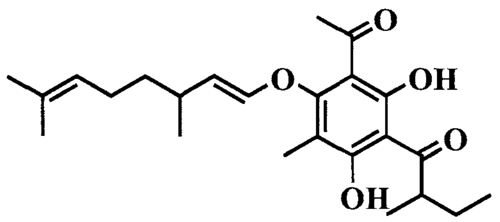



**Fig. (9).** Chemical structure of 2-acetyl-3,5-dihydroxy-1-geranoxy-6-methyl-4-(2-methyl) butyryl-benzene from *H. japonicum*.

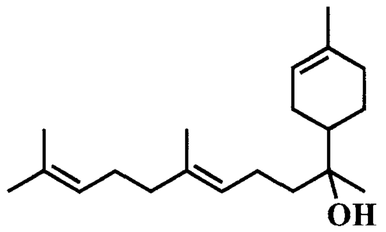



**Fig. (10).** Chemical structure of 9-geranyl-α-terpineol.

The occurrence of monocyclic diterpenes with a prenylbisabolane skeleton is quite uncommon and it is regarded as an interesting chemotaxonomic feature of this plant species.

However, the structural combination of the phloroglucinol and terpenoid moieties appears to be a biosynthetic peculiarity of the *Hypericum* plants.

Recently, other new structural types of prenylated phloroglucinols have been isolated from the aerial parts of *H. papuanum*, traditionally used in Papua New Guinea for the treatment of sores [59]. Among the new compounds obtained from this plant, the tricyclic acylphloroglucinols, papuaforins A-E, Fig. (**11**), should especially be mentioned.

They resemble hyperforin in their chemical structure, having in addition a 2,2-dimethyl-2*H*-pyran ring, most probably derived by the cyclization of a 3-methylbut-2-enyl side chain with an enolic hydroxyl group. Only a few reports describe a similar natural tricyclic system [59-60].

New compounds from the genus continue to emerge: nine new polyprenylated benzoylphloroglucinols, named hyperibones A-I, have been recently isolated from the aerial parts of *H. scabrum* [61].

## Flavonoids

The occurrence of flavonoids in the methanolic extract from the aerial parts of *H. perforatum* is well documented [3,9,10,22]. The content of these metabolites is reported to be high in the flowers during the budding stage, immediately before flowering (up to 12%/dry weight) [22,62]. Nevertheless, flavonoid concentration in St. John's Wort is reported to be influenced by the altitude, being higher in plants growing at higher altitudes.

The flavonol quercetin and its glycoside derivatives, rutin, hyperoside, isoquercitrin, quercitrin, and the biflavones I3,II8-biapigenin and amentoflavone are described as the most common in the drug extracts, Fig. (**12**). Recently, a detailed chemical study by on-line coupling of HPLC with UV, NMR and MS [63], allowed the determination of two other flavonoids in *H. perforatum*: the quercetin-galacturonide and the quercetin-arabinoside.

In a phytochemical study of *Hypericum* species growing in Slovenia [64], it has been reported that the rutin content in *H. perforatum* is positively correlated with the altitude of the locality where the plant grows.

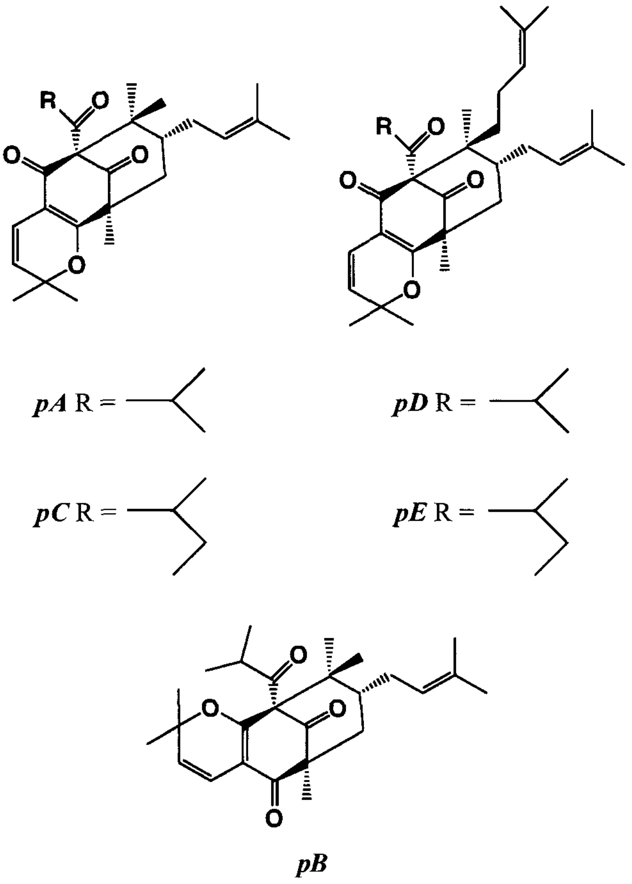
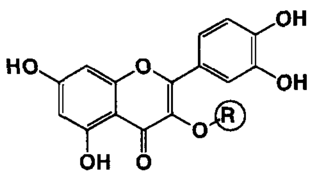
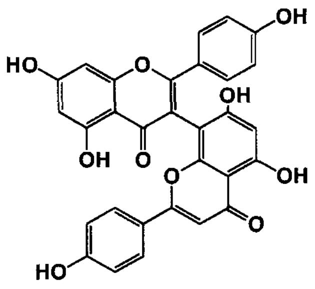



**Fig. (11).** Chemical structure of papuaforins (*p*)*A-E*.

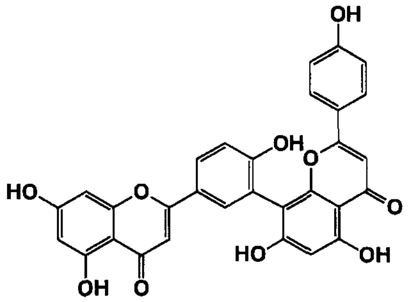



| R | Flavonoids |
|---|---|
| H | Quercetin |
| α-L-Rha | Quercitrin |
| β-D-Glc | Isoquercitrin |
| β-D-Gal | Hyperoside |
| β-D-Glc (6<--1)α-L-Rha | Rutin |

**Fig. (12)** Flavonoids from *H.perforatum.*




I3,II8-Biapigenin




Amentoflavone
(I3',II8-Biapigenin)

A chemotaxonomic study [65] on *H. perforatum* populations from Italy revealed the existence of a chemotype characterized by the absence of rutin. Our investigation of the chemical profile of St. John's Wort plants collected in various localities in South Italy [66,67] also confirms the presence of a specific chemical race which does not produce rutin. However, in those studies, a possible correlation between the content of rutin and the altitude was not looked for.

A compositional analysis of flavonoids from other species of *Hypericum*, namely *H. hirsutum*, *H. maculatum*, *H. tatrapterum*, *H. montanum* and *H. humifusum* [64], *H. patulum* and *H. olympicum* [68], has also been carried out.

A differentiation between the above species has been possible based on the relative percentage of amentoflavone. The highest amount of the biflavone has been detected in *H. montanum* and *H. hirsutum*, amounting to 2.5-3 times more than in *H. perforatum.*

Attempts to produce flavonoids by *in vitro* cultures of *H. perforatum* led to variable results. Some Authors obtained flavonoids of the same type of those synthesized *in vivo* by *H. perforatum*, while others report on the isolation of flavonoids derived from luteolin, not described *in vivo* in plants of *H. perforatum* [69,70].

## Xanthones

Xanthones are quite widespread in the *Clusiaceae* and several types have been isolated within the genus *Hypericum* [71].

Co-occurrence of simply oxygenated xanthones and more complex prenylated structures has been described in the genus and the 1,3,5,6-oxygenation pattern appears to be the most common for the alkylated xanthones [72].

Xanthones have been identified in different species, such as *H. perforatum* [9,10,22], *H. inodorum*, *H. calycinum* and *H. roeperanum* [72], mainly in the underground organs.

Figure (**13**) shows two examples of xanthones produced by hypericum plants. Metabolite *1*, calycinoxanthon D (1,3,5,6-tetrahydroxy-4-lavandulylxanthone), isolated from *H. calycinum* and *H. roeperanum* [72], and compound *2*, roeperanone (1,3,5,6-tetrahydroxy-4-trans-sesquilavandulylxanthone), identified in the roots of *H. roeperanum*, represent xanthones bearing a terpene alkyl moiety.

Roeperanone is the first xanthone identified in plants, substituted with a sesquiterpene alkyl group.

The compound depicted in Fig. (**14**), 1,3,5,6-tetrahydroxy-2-(2',2'-dimethyl-4'-isoprenyl)-cyclopentanylxanthone (5-O-demethyl paxanthonin) has also been isolated for the first time from *H. roeperanum* [72].

The xanthone shows the usual 1,3,5,6-oxygenation pattern, but its structure is characterized by an unusual five-membered ring, probably due to the cyclization of a monoterpene unit.

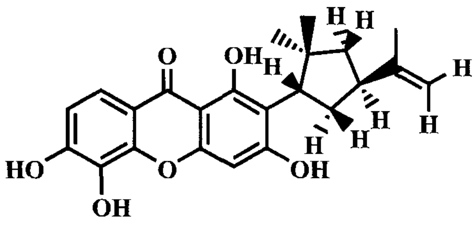
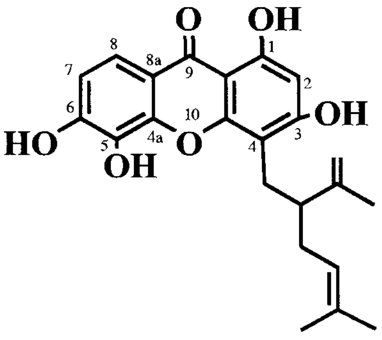


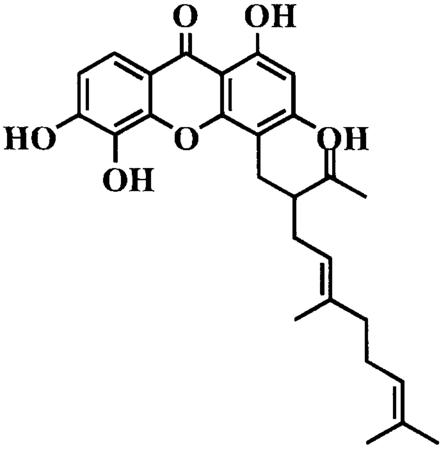

**Fig. (14).** Chemical structure of 5-O-demethyl-paxanthonin.

Two other xanthones, have been isolated from the same species of *Hypericum* [72]: *a*) 2-deprenylrheediaxanthone B (1,5,6 - trihydroxy-4',5'-dihydro - 4', 4' , 5'- trimethylfurano - ( 2', 3': 3, 4 ) xanthone) and *b*) isojacareubin (1,5,6 - rihydroxy - 6',6'-dimethylpyrano - (2',3':3,4) - xanthone). They were peculiar in their structure, being conjugated with a furan and a pyran ring, respectively.

Several xanthones have also been isolated from the aerial organs of *H. ascyron* [73], a Chinese natural drug used in the treatment of headache, nausea and abscesses.




*1*




*2*

**Fig. (13).** *1)* Calycinoxanthon D; *2)* Roeperanone.

The new 3,6-dihydroxy-1,7-dimethoxyxanthone was identified in this plant together with the 5-chloro-1,6-dihydroxy-3-methoxy-8-methylxanthone, *1)*, *2)*, Fig.(**15**), which is the first chloroxanthone isolated from higher plants.

New oxygenated xanthones were characterized from heartwood, root and leaf extracts of *H. geminiflorum* [74,75], namely *a)* 2, 3-dihydroxy-1,6,7-trimethoxy-xanthone; *b*) 3,6-dihydroxy-1,5.7-trimethoxy-xanthone; *c*) 6,7-dihydroxy-1,3-dimethoxy-xanthone; *d*) 4-hydroxy-1,2-dimethoxy-xanthone and *e*) gemixanthone A, *3)* Fig. (**15**). The last compound represents a xantholignoid with a *trans*-substituted 1.4-dioxane ring between the xanthone moiety and the phenyl group.

Several studies [76-78] have shown that xanthones are the main metabolites synthesized *in vitro* by cell cultures from different species of *Hypericum*, even though they were not present *in vivo* in the parent plants.

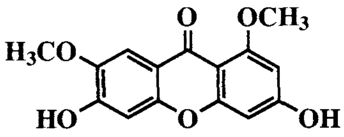
During an investigation (see above) on the ability of *in vitro* cultures from *H. perforatum* to produce the active principles, hypericins and hyperforins, it was found [37,79] that the extracts from cell suspensions and calli contained instead high amounts of xanthones.

To the best of our knowledge, the occurrence *in vivo* of this chemical class of compounds is low in St. Jonh's Wort. In fact, only kielcorin (0.01%) and the 1,3,6,7-tetrahydroxy-xanthone (trace amounts) have been so far isolated from its roots and its leaves and stems, respectively [9].

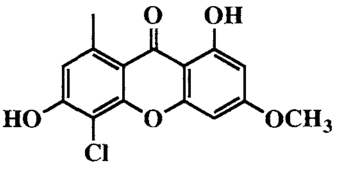
The *in vitro* mixture of xanthones produced by *H. perforatum* mainly contained paxanthone [79], never isolated from the species before, but firstly characterized in cell cultures of *H. patulum* [76].

## Benzophenones

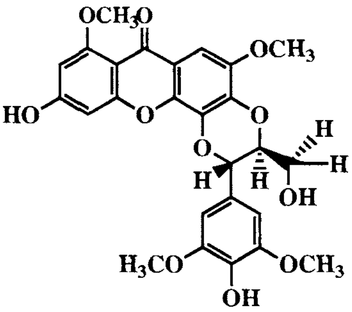
Benzophenones represent a further example of metabolites synthesized within the genus *Hypericum*. They are indicated as the biogenetic precursors of xanthones, therefore their occurrence in *Hypericum* species, producing various structural types of xanthones, should not be regarded as unexpected. Investigation of the chemical composition of the aerial parts of *H. sampsonii*, a Chinese medicinal plant used against diarrhoea, swellings and snakebites has led to the structural elucidation of a series of polyprenylated benzophenones [80-82], named sampsoniones A-J, possessing a unique




*1*




*2*




*3*

**Fig. (15).** Examples of xanthones from *Hypericum* sp.

caged carbotetracyclo skeleton, with an adamantyl core, derived by a complex cyclization of prenyl substituents, Fig. (**16**). Sampsoniones were shown to have cytotoxic activity.

In contrast to prenylated benzophenones, simple benzophenones are of rare occurrence in the *Clusiaceae* family and so far only few examples have been described in the literature [83].

Extraction of the aerial organs of *H. annulatum* allowed the isolation of hyperiphenonoside (2'-*O*-ß-D-glucopyranosyl-2,4,5',6-tetrahydroxybenzophenone), the second *O*-glycosilated benzophenone isolated from plants, and annulatophenone (2, 3',5',6'-tetrahydroxy-4-methoxyphenone) [83].

More recently, from the same species of *Hypericum,* other two glycosilated benzophenones, annulatophenonoside and acetylannulatophenonoside, have been characterized: both compounds are conjugated with a unit of arabinose [84].

## BIOACTIVITY

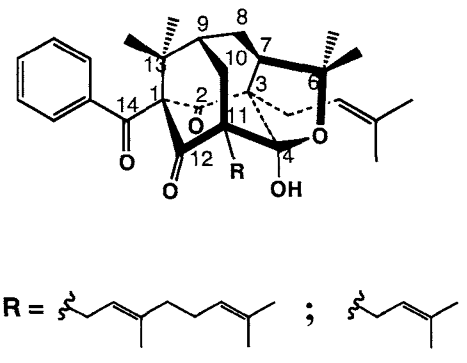
### Antidepressant activity

Herbal preparations from the dried aerial parts of *H. perforatum* are currently used for the treatment of mild to moderate depression [4,5]. The antidepressant effectiveness of the plant is well documented by several studies [3,10,85-87], which also indicate that the drug has advantages compared to synthetic antidepressants.

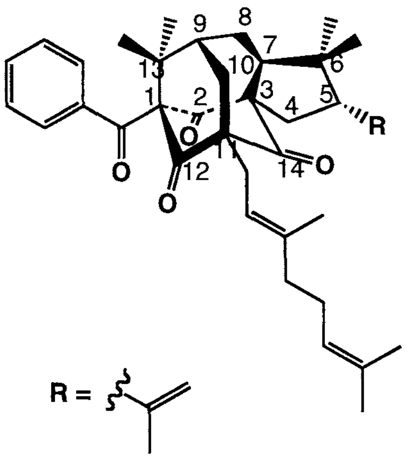
Nevertheless, the active principle(s), the mechanism of action and the pharmacology still remain to be fully understood.

Quality of the drug extracts (60 % ethanol or 80% methanol) is usually standardized by the total content of hypericins (0.3%), determined by spectrophotometric measurement [34,38].

Although the alcoholic extract of the drug contains several other constituents, as usually determined by HPLC, Fig. (**17**), hypericin has for long time been reported as the only candidate responsible for the antidepressant activity [3,86,87].



Sampsoniones A and B



Sampsonione D

**Fig.(16)**. Chemical structure of sampsoniones from *H. sampsonii*.

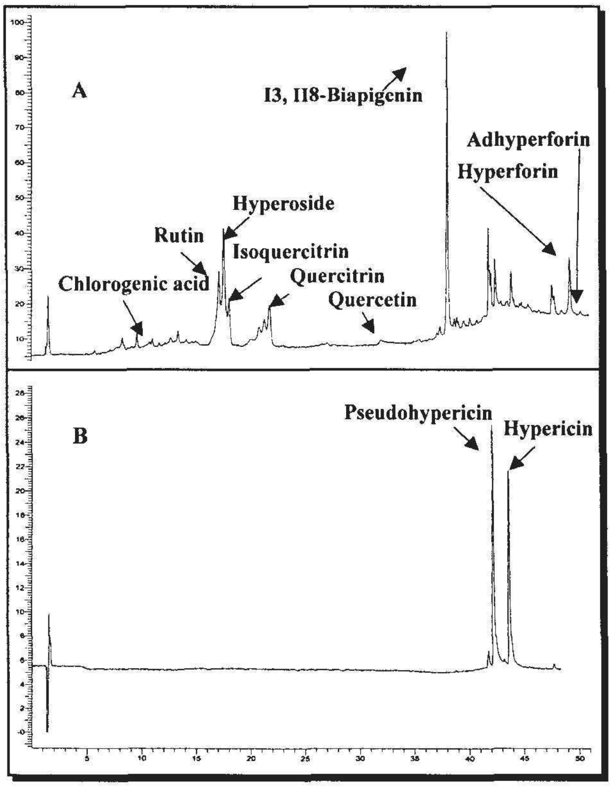



**Fig. (17).** HPLC chromatograms of *H. perforatum* at 270 nm (A) and 590 nm (B).

The mechanism of action generally attributed to hypericin consists with the inhibition of monoaminooxidases (MAO) and/or cathecol-O-methyltransferases (COMT) [10,3,85,86]. However, St. John's Wort extracts devoid of hypericin have been found to retain their antidepressant efficacy [4,86] and some studies reported only weak inhibition of MAO activity by hypericum extracts [86,88].

Other studies have shown that hypericum extracts inhibit *in vitro* the re-uptake of monoamine neurotransmitters such as serotonin, dopamine and noradrenaline [10,87].

More recent investigations on the therapeutic features of *H. perforatum* suggest that hyperforin is the main antidepressant constituent of the drug [3,5].

Assays with extracts devoid of hypericin but with a high content of hyperforin as well as assays with pure hyperforin demonstrated that this metabolite can modulate the function of various neurotransmitters involved in depression, being a potent inhibitor of serotonin, dopamine, noradrenaline and GABA uptake with almost equal potency. Moreover, results from clinical studies evidenced that the antidepressant efficacy of *Hypericum* preparations correlates with their content of hyperforin [6,87].

In a specific work, it has been shown that chemical modification of hyperforin by acylation, oxidation and alkylation produce detrimental effects on the molecule activity as inhibitor of serotonin uptake [89], and the presence of the enolized cyclohexanedione moiety in its structure has been related to its efficacy on the re-uptake of neurotransmitters.

Today, the current opinion is that more than one active metabolite contributes to the antidepressant usefulness of *H. perforatum* and each of them with its distinct mode of action.

Thus, pure adhyperforin has been found to have the same inhibitorial potency as hyperforin on the uptake of monoamine neurotransmitters [86], and more polar drug constituents such as flavonoid glycosides and procyanidins [3,87,90] have also been shown to be effective in behavioral models of depression.

## Antiviral activity

Evidence of antiviral effects has especially been shown for St. John's Wort [2,3,10]. Plant preparations with different chemical composition have been assayed against various viruses and both, hypericin and pseudohypericin, were found to be particularly effective as virucidal agents. The two compounds have been shown to be active against a broad range of viruses and retroviruses, such as herpes simplex virus types I and II, vesicular stomatitis and influenza viruses, cytomegalovyrus and human immunodeficiency virus-1 [2,3,10,91,92].

Studies on the mechanism of action, suggested that hypericin antiviral effect is directed towards enveloped viruses, while non enveloped viruses, such as adenovirus and poliovirus, are unaffected [4]. Hypericin virucidal effect is increased by light, however its antiviral properties against selected viruses [7] have also been documented in the dark.

Virus inactivation by hypericin seems to involve inhibition of various stages of virus replication depending on the integrity of the viral envelope. Photoactivation of hypericin leads to the formation of active oxygen species that likely react with viral components making them unable to assembly into complete virus particles.

In addition, some Authors suggested that the antiretroviral efficacy of hypericin is associated with the inhibition of protein-kinase C-mediated phosphorylation, occurring during the viral infection of the cells [10].

Nevertheless, hypericin is the first photodynamic metabolite which has been shown to have virucidal efficacy *in vivo* in experimental animals [2,92].

## Antimicrobial activity

The two preparations from *H. perforatum*, novoimanine, the 0.25% aqueous alcoholic solution, and imanine, the water soluble fraction showed antibacterial properties against *Staphylococcus aureus* [93]. Chemical analysis of novoimanine showed that the main extractives consisted with phenolic glycosides of low polarity [94].

Hyperforin has also been reported as a component of this preparation [95], being 5-fold more active than novoimanine. The antimicrobial effects of *H. perforatum*, since then, have been attributed to hyperforin, the main acylphloroglucinol constituent [3,8]. This compound showed antibacterial activity especially against Gram-positive bacteria, such as for example *S. aureus*, *Bacillus subtilis* and *B. cereus*, while no inhibitorial effect has been so far evidenced on the growth of Gram-negative bacteria and yeasts. The chemical instability of hyperforin has however limited the investigations of its biocide activity. Moreover, it has been observed that high concentrations are needed for its antimicrobial activity [3,8].

Historically, *H. perforatum* is a well known traditional remedy against infected wounds and skin lesions, and this effect is in part attributed to the antimicrobial activity of the drug. In a recent investigation on the antimicrobial effect of St. John's Wort [96,97], it was demonstrated that hypericin should also be regarded as an active antibiotic ingredient of *H. perforatum* extracts. Comparison of inhibitory activities showed that, in general, hypericin had lower minimum inhibitory concentrations - MICs (12.5 $\gamma$) than hyperforin.

The wound-healing effect of St. John's Wort has also been attributed to flavonoids [22]. A commercial preparation containing 0.412% of quercitrin

was reported to be highly effective against *S. aureus*. However, during our study [96,97] we could not prove any inhibitory activity of flavonoids,.

Important antimicrobial activity is also described for phloroglucinol derivatives other than hyperforin. Thus, some of the phloroglucinols isolated from *H. japonicum* [55] have been assayed against Gram-positive bacteria and found to be particularly effective against *S. aureus*. The presence of a benzoyl group on the filicinic acid moiety, typical of their structure, seems to be essential for their bioactivity.

Similarly, the phloroglucinols, drummondins A-C, isolated from *H. drummondii* [53], showed significant growth inhibitorial activity against *S. aureus*, *B. subtilis* and *Mycobacterium smegmatis*.

Moreover, phloroglucinols from *H. brasiliense,* also containing a filicin acid moiety in their structure, have been found effective against *B. subtilis* [54].

Antimicrobial potential of different extracts prepared from the aerial parts of *H. mysorense* and *H. patulum*, endemic of India, has been recently evaluated [98] and both species have been shown to be active, to various extent, against some Gram-positive (*B. subtilis*, *S. aureus*, *B. coagulans, B. megatorium*) as well as against some Gram-negative (*P. cepacia* and *E. coli*) bacteria. No indication of the active compounds was however given on those studies. Antifungal activity against *C. albicans* has been found for some xanthones isolated from *H. roeperanum* [72].

## CONCLUDING REMARKS

The great deal of investigations and scientific reports on the chemistry and biological properties of plants belonging to the genus *Hypericum* have disclosed characteristic and sometimes unique features.

Until now, the main interest has been addressed to *H. perforatum*, which is a leading drug for depression treatment in Europe as well as in the USA, being marketed not only as herbal medicinal products but also in the form of dietary supplements.

However, the available scientific literature indicates that other species are also important as a source of new chemical types, which might have potential as therapeutic agents. Moreover, the variety of molecules identified makes the *Hypericum* species an interesting and suitable model to investigate biosynthetic relationships between classes of metabolites.

## REFERENCES


[1] Judd, W.S.; Campbell, C.S.; Kellogg, E.A.; Stevens, P.F. *Plant Systematics – A Phylogenetic Approach*, Sinauer Assoc., Inc. USA, **1999.**

[2] Lavie, G.; Mazur, Y.; Lavie, D.; Meruelo, D. *Med. Res. Rev.*, **1995**, *15,* 111-119.

[3] Barnes, J.; Anderson, L.A.; Phillipson, J.D. *J. Pharm. Pharmacol.*, **2001**, *53*, 583-600.

[4] Chatterjee, S.S; Bhattacharya, S.K; Wonnemann, M.; Singer, A.; Muller, W.E *Life Sci.*, **1998**, *63*, 499-510.

[5] Chatterjee, S.S.; Noldner, M.; Koch, E.; Erdelmeier, C. *Pharmacopsychiatry,* **1998**, *31* (Suppl.), 7-15.

[6] Laakmann, G.; Schüle, C.; Baghai, T.; Keiser, M. *Pharmacopsychiatry,* **1998**, *31* (Suppl.), 54 -59.

[7] Hudson, J.B.; Lopez-Bazzocchi, I.; Towers, G.N.H. *Antiviral Res.*, **1991**, 15: 101-112.

[8] Reichling, J.; Weseler, A.; Saller, R.A. *Pharmacopsychiatry,* **2001**, *34* (Suppl.), 116 -118.

[9] Nahrstedt, A.; Butterweck, V. *Pharmacopsychiatry,* **1997**, *30*, 129-134.

[10] Bombardelli, E.; Morazzoni, P. *Fitoterapia,* **1995**, *LXVI*, 43 - 98.

[11] Tutin, T.G.; Heywood, V.H.; Burges, N.A.; Moore, D.M.; Valentine, D.H.; Walters, S.H.; Webb, D.A. In *Flora Europaea,* University Press: Cambridge, **1968**, Vol. 2, pp. 261-269.

[12] Robson, N.K.B. *Bull. Brit. Mus. (Nat. Hist.), Bot.,***1981**, 8, 72; 176-205.

[13] Cappelletti, E.M. In *Iperico* – Aggiornamenti Scientifici, Studio Edizioni, *Suppl. Erboristeria Domani*, **2002**, 256, 9-16.

[14] Pignatti, S. In *Flora d'Italia*, Edagricole: Bologna, **1982**, pp. 343-351.

[15] Mathis , C.; Ourisson, G. *Phytochemistry*, **1964**, *3*, 115-131.

[16] Benigni, R.; Capra, C.; Cattorini, P.E. Piante Medicinali: Chimica, Farmacologia e Terapia, Milano, Inverni & Della Beffa, **1971**.

[17] Mathis , C.; Ourisson, G. *Phytochemistry*, **1964**, *3*, 133-141.

[18] Mathis , C.; Ourisson, G. *Phytochemistry*, **1964**, *3*, 377-378.

[19] Mathis , C.; Ourisson, G. *Phytochemistry*, **1964**, *3*, 379.

[20] Brondz , I.; Greibrokk, T.; Aasen, A.-J. *Phytochemistry*, **1983**, *22*, 295-296.

[21] Brondz , I.; Greibrokk, T. *J. Nat. Prod.*, **1983**, *46*, 940-941.

[22] American Herbal Pharmacopoeia and Therapeutic Compendium – St. John's Wort- *Hypericum perforatum*, **1997**.
[23] Nogueira, T.; Duarte, F.; Venancio, F.; Tavares, R.; Lousa, M.; Bicchi, C.; Rubiolo, P. *Silva Lusitana*, **1998**, *6*, 55-61.
[24] Gudzic, B.; Dordevic, S.; Palic, R.; Stojanovic, G. *Flavour Fragarance J.*, **2001**, *16*, 201-203.
[25] Couladis, M.; Baziou, P.; Petrakis, P.V.; Harvala, C. *Flavour Fragarance J.*, **2001**, *16*, 204-206.
[26] Sajjadi, S.; Rahiminezhad, M.R.; Mehregan, I.; Poorassar, A. *J. Essent. Oil Res.*, **2001**, *13*, 43-44.
[27] Avato, P., unpublished
[28] Baser, K.H.C.; Ozek, T.; Nuriddinov, H.R.; Demirci, A.B. *Chem. Nat. Comp.*, **2002**, *38*, 54-57.
[29] Bertoli, A.; Pistelli, L.; Morelli, I.; Spinelli, G.; Menichini, F. *J. Essent. Oil Res.*, **2000**, *12*, 617-620,
[30] Knox, J.P.; Dodge, A.D. *Plant Cell Environ.*, **1985**, *8*, 19-25.
[31] Brockmann, H.; Sanne, W. *Naturwiss.*, **1953**, *40*, 509.
[32] Brockmann, H.;, Kluge, F.; Muxfeldt, H. *Chem. Ber.*, **1957**, *90*, 2302.
[33] Arabei, S.M.; Galaup, J.P.; Jardon, P. *Chem. Phys. Lett.*, **1997**, *270*, 31-36.
[34] Brolis, M.; Gabetta, N., Fuzzati, R.; Pace, R.; Panzeri, F.; Peterlongo, F. *J Chromatog* , **1998**, *25*,9-16.
[35] Rodelwald, G.; Arnold, R.; Griesler, J.; Steglich, W. *Angew. Chem.*, Int. Ed. Engl. **1977**, *16*, 46-47.
[36] Spitzner, D. *Angew. Chem.*, Int. Ed. Engl., **1977**, *16*, 46.
[37] Avato, P.; Argentieri, M.P.; Pasqua, G.; Monacelli, B.; Ferrari, F. Atti 11° Congresso Nazionale della Società Italiana di Fitochimica, P06, **2002**.
[38] Deutscher Arzneimittel Codex (DAC), **1986**. (Ergänzung zum Arzneibuch), Govi Verlag, Frankfurt/Main (in der Ergänzung von 1991).
[39] Holz, J.; Ostrowoski, E. *Dtsch. Apoth. Ztg.*, **1987**, *23*, 1227-1230.
[40] Southwell, I.A.; Campbell, M.H. *Phytochemistry,* **1991**, *30,* 475-478.
[41] Southwell, I.A.; Bourke, C.A. *Phytochemistry,* **2001**, *56*, 437-441.
[42] Delaey, E.M.; Kamuhabwa, R.A.; Vandenbogaerde, A.L.; de Witte, P. A. *Planta Med.*, **1999**, *65*, 719-722.
[43] Mathis, C.; Ourisson, G. *Phytochemistry*, **1963**, *2*, 157-171.
[44] Kitanov, G.M. *Biochem. Syst. Ecol.*, **2001**, *29*, 171-178.

[45] Erdelmeier, C.A.J. *Pharmacopsychiatry,* **1998**, *31* (Suppl.) 2-6.
[46] Bystrov, N.S.; Chernov, B.K.; Dobrynin, V.N.; Kolosov, M.N. *Tetrahedron Lett.*, **1975**, *32*, 2791-2794.
[47] Orth, H.C.J.; Rentel, C.; Schmidt, P.C. *J. Pharm. Pharmacol.,* **1999**, *51,* 193-200.
[48] Fuzzati, N.; Gabetta, B.; Strepponi, I.; Villa, F. *J. Chromatogr.* A, **2001**, *926*, 187-198.
[49] Ruecker, G.; Manns, D.; Hartmann, R.; Bonsels, U. *Arch. Pharm.*, **1995**, *328*, 725-730.
[50] Trifunovic, S.; Vajs, V.; Macura, S.; Juranic, N.; Djarmati, Z.; Jankov, R.; Milosavljevic, S. *Phytochemistry*, **1998**, *49*, 1305-1310.
[51] Verotta, L.; Appendino, G.; Jakupovic, J.; Bombardelli, E. *J. Nat. Prod.,* **2000**, *63,* 412 -415.
[52] De Shan, M.; Hu, L.H.; Chen, Z.L. *J. Nat. Prod.*, **2001**, *64*, 127-130.
[53] Jayasuriya, H.; McChesney, J.D.; Swanson, S.M.; Pezzuto, J.M. *J. Nat. Prod.*, **1989**, *52*, 325-331.
[54] Rocha, L.; Marston, A.; Potterat, O.; Kaplan, M.A.C.; Hostettman, K. *Phytochemistry*, **1996,** *43*, 185-188.
[55] Ishiguro, K.; Yamaki. M.; Kashihara, M.; Takagi, S.; Isoi, K. *Planta Med.*, **1990**, *56*, 274-276.
[56] Ishiguro, K.; Nagata, S.; Fukumoto, H.; Yamaki, M.; Isoi, K. *Phytochemistry*, **1994**, *35*, 469-471.
[57] Hu, L-H.; Khoo, C-W.; Vittal, J.J.; Sim, K-Y. *Phytochemistry*, **2000,** *53*, 705-709.
[58] Ishiguro, K.; Nagareya, N.; Fukumoto, H. *Phytochemistry*, **1998**, *47*, 1041-1043.
[59] Winkelmann, K.; Heilmann, J.; Zerbe, O.; Rali, T.; Sticher, O. *J. Nat. Prod.*, **2001**, *64*, 701-706.
[60] Henry, G.E.; Jacobs, H.; Carrington, S.; McLean, S.; Reynolds, W.F. *Tetrahedron*, **1999**, *55*, 1581-1596.
[61] Matsuhisa, M.; Shikishima, Y.; Takaishi, Y.; Honda, G.; Ito, M.; Takeda, Y.; Shibata, H.; Higuti, T.; Kodzhimatov, O.; Ashurmetov, O. *J. Nat. Prod.*, **2002**, *65*, 290-294.
[62] Tekel'ová, D.; Repcak, M.; Zemkove, E.; Toth, J. *Planta Med.*, **2000,** *66*, 778-780.
[63] Hansen, S.H.; Jensen, A.G.; Cornett, C.; Bjornsdottir, I.; Taylor, S.; Wright, B.; Wilson, I.D. *Anal. Chem.*, **1999**, *71*, 5235-5241.
[64] Umek, A.; Kreft, S.; Kartnig, T.; Heydel, B. *Planta Med.*, **1999,** *65*, 388-390.

[65] Mártonfi, P.; Gabetta, N.; Miroslav, R.; Ciccarelli, D.; Garbari, F. *Biochem. Syst. Ecol.*, **2001**, *29,* 659-661.
[66] Avato, P.; Guglielmi, G. First International Congress on Plant Metabolomics, Book of Abstracts, P18, **2002**.
[67] Avato, P.; Guglielmi, G. *Pharm. Biol.*, **2002**, submitted.
[68] Baureuthel, K.; Buter, B.; Engesser, K.; A.; Burkard, W.; Scaffner, W. Pharm. Acta Helv., **1997**, *72*, 153-157.
[69] Dias, A.C.P.; Tomás-Barberán, F.A.; Fernandes-Ferreira, M.; Ferreres, F. *Phytochemistry*, **1998**, *48*, 1165-1168.
[70] Kartnig, T.; Göbel, I.; Heydel, B. *Planta Med.*, **1996**, 62, 51-53.
[71] Bennet, G.J.; Lee, H.H. *Phytochemistry*, **1989**, *28*, 967-998.
[72] Rath, G.; Potterat, O.; Mavi, S.; Hostettmann, K. *Phytochemistry*, **1996**, *43*, 513-520.
[73] Hu, L-H.; Yip, S-C.; Sim, K-Y. *Phytochemistry*, **1999**, *53*, 1371-1373.
[74] Chung, M-I.; Weng, J-R.; Lai, M-H.; Yen, M-H.; Lin, C-N. *J. Nat. Prod.*, **1999**, *62*, 1033-1035.
[75] Chung, M-I.; Weng, J-R.; Teng, J-P.; Lin, C-N. *Planta Med.*, **2002**, *68*, 25-29.
[76] Ishiguro, K.; Nakajima, M.; Fukumoto, H.; Isoi, K. *Phytochemistry*, **1995**, *38*, 867-869.
[77] Ishiguro, K.; Yamamoto, R.; Oku, H. J. Nat. Prod., **1999**, *62*, 906-908.
[78] Dias, A.C.P.; Seabra, P.B.; Andrade, P.B.; Ferreres, F.; Fernandes-Ferreira, M. *Plant Sci.*, **2000**, *150*, 93-101.
[79] Pasqua, G.; Monacelli, B.; Ferrari, F.; Mares, D.; Santamaria, A.R.; Argentieri, M.; Avato, P. Atti 97° Congresso Società Botanica Italiana, pg.18, **2002**.
[80] Hu, L-H.; Sim, K-Y. *Tetrahedron Lett.*, **1998**, *39*, 7999-8002.
[81] Hu, L-H.; Sim, K-Y. *Tetrahedron Lett.*, **1999**, *40*, 759-762.
[82] Hu, L-H.; Sim, K-Y. *Organic Lett.*, **1999**, *1*, 879-882.
[83] Kitanov, G.M.; Nedialkov, P.T. *Phytochemistry*, **2001**, *57*, 1237-1243.
[84] Nedialkov, P.T.; Kitanov, G.M. *Phytochemistry*, **2002**, *59*, 867-871.
[85] Kasper S. *Pharmacopsychiatry*, **2001**, *34*, S51-S55.
[86] Di Carlo, G.; Borrelli, F.; Ernst, E.; Izzo, A.A. *Trends in Pharm. Sci.,* **2001**, *22*, 292-297.
[87] Jensen, A. G.; Hansen, S.H.; Nielsen, E.Ø. *Life Sci.*, **2001**, *68*, 1593-1605.

[88] Müller, W.E.; Rolli, M.; Schafer, C.; Hafner, U. *Pharmacopsychiatry*, **1997**, *30* (Suppl.), 129-134.

[89] Verotta, L.; Appendino, G,; Belloro, E.; Bianchi, F., Sterner, O.; Lovati, M.; Bombardelli, E. *J. Nat. Prod.*, **2002**, *65*, 433-8.

[90] Butterweck, V., Jürgenliemk, G.; Nahrstedt A.; Winterhoff, H. *Planta Med.*, **2000**, *66*, 3-6.

[91] Wood, S.; Huffman, J.; Weber, N.; Andersen, D.; North, J.; Murray, B.; Sidwell, R.; Hughes, B. *Planta Med.*, **1990**, *56*, 651-652.

[92] Meruelo, D.; Lavie, G.; Lavie, D. *Proc. Natl. Acad. Sci.* **1988**, *85*, 5230-5234.

[93] Negrash, A.K.; Pochinok, P.Ya. Fitontsidy, Mater. Soveschch., 6th Meeting, **1972**, 198-200. Publisher: "Naukova Dumka", Kiev, USSR.

[94] Maksyutina, N.; Koget, T.A. *Khim. Prir. Soedin.,***1971** 7: 363-367.

[95] Derbentseva, N.A.; Aizenman, B.Yu.; Garagulya, O.O. *Mikrobiol. Zh.* (Kiev), **1971**, *33*, 569-572.

[96] Avato, P.; Raffo , F.; Guglielmi, G.; Vitali, C.; Rosato, A. International Symposium on "Leads Compounds from Higher Plants", Abstract Book, P064, **2001**.

[97] Avato, P.; Raffo, F.; Guglielmi, G.; Vitali, C.; Rosato, A. *Phytother. Res.*, **2002**, submitted.

[98] Mukherjee, P.; Saritha, G.S.; Suresh, B. *Phytother. Res.*, **2002**, *16*, 692-695.

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# BIOACTIVE TRITERPENES AND RELATED COMPOUNDS FROM CELASTRACEAE

*Dedicated to Prof. Antonio G. González, in memoriam*

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**ABSTRACT:** The Celastraceae family is a source of important bioactive secondary metabolites. Among the compounds isolated from their species, triterpenes and triterpenoid quinonemethides are of great interest, due to the wide range of biological activities showed by those compounds. At the present time, great interest had been devoted to the study of the species of the family searching for new candidates or prototypes of drugs than could contribute to the fight against diseases like AIDS and cancer. The isolated compounds show mainly antimicrobial, cytotoxic, antitumoral, antiviral, antiinflamatory, hepatoprotective, antifeedant and insecticidal activities. This review covers the literature from 1997 up to August 2002 and contains discussions on chemistry and biological activity of triterpenes, triterpenoid quinonemethides and other related compounds. Emphasis has been dedicated to compounds that shown some kind of biological activity and with structure – activity relationships if any. The high potential of these compounds as therapeutical agents is also emphasized.

## INTRODUCTION

For centuries, mankind has searched for the cure of illnesses in herbs. In that sense, approximately one third of drugs actually in use are from plant origin [1]. In the last decades, however, most of the drugs incorporated to the therapeutic arsenal come from synthetic sources. Nevertheless, in recent years a remarkable interest in the study of medicinal plants has appeared, mainly due to the concern about the destruction of rain forest habitats, with the risk of extinction of numerous species. Another fact is the loss of the knowledge related to the use of plant remedies by the people from native settlements, that is very common when they are incorporated to the urban life, and/or when their environmental conditions are seriously transformed.

Plants of the Celastraceae family had been used in folk medicine of various countries [2]. From the species of this family, sesquiterpenes,

triterpenes and triterpenoid quinonemethides that showed antifeedant, insecticide, antimicrobial, cytotoxic, hepatoprotective and antimalarial activities had been isolated [3,4].

Triterpenes belong to a wide group of compounds frequently isolated from plant sources. The biosynthetic pathways of many of the skeletons are known and the range of biological activities makes them attractive as potential new drugs.

Among the compounds reported, mainly oleanes, lupanes, friedo-oleanes, glutinanes, taraxeranes, ursanes and dammaranes had been isolated from Celastraceae species.

Triterpenoid quinonemethide compounds conform a small group of D:A-*friedo-nor*-oleanane derivatives that invariably contain oxygenated functionalities at carbons 2 and 3. These compounds are found only in Celastraceae and Hippocrateaceae and are considered as chemotaxonomic indicators [5]. Within the last 10 years, another group of compounds derived from quinonemethide triterpenes appeared in the literature. These compounds are dimers and trimers, derived from pristimerin, tingenone and/or their congeners, joined by two ether linkages between the two A rings in dimers and by an additional ether linkage between B' and A'' rings in trimers [6,8].

Since the isolation of celastrol in 1936 by Chinese workers a variety of quinonemethides have been encountered in Celastraceae species and their structures and a wide range of biological activities have been reported thus far, resulting quite interesting activities against microorganisms and tumor cells [9,11].

## CHEMOTAXONOMY

The Celastraceae family, named commonly as the bittersweet family, consists of 50-55 genera with 800-850 species with pantropical distribution. Nevertheless, a fair number of species are found in temperate regions. The largest genera are *Maytenus* (225 species), *Euonymus* (200 species), *Cassine* (40 species), and *Celastrus* (30 species) [12, 13].

Hippocrateaceae consist of only two genera, *Salacia* (200 species) and *Hippocratea* (100 species) with species widely distributed in tropical regions [13]. For some taxonomist, Hippocrateaceae should be included into Celastraceae whilst others maintain that it should be considered a different family. According to Robson, Hippocrateaceae are a not natural

group, but derived in two separate lines from Celastraceae [14]. Heywood has included the species of Hippocrateaceae into Celastraceae [12]. The amalgamation of the two families is justified by the presence of some common chemotaxonomic markers as dulcitol, polyisoprene and triterpenoid quinonemethides [5]. In a recent work, using phytocrome B gene sequences Simmons *et. al* found that Hippocrateaceae are resolved as a monophyletic group (albeit poorly supported) within the paraphyletic Celastraceae s.s. This resolution supports the taxonomic inclusion of Hippocrateaceae within Celastraceae [15].

Celastraceae is a large family comprising trees, shrubs and woody lianas with a Gondwanan distribution. The classification at the generic level has undergone changes due to hibridization [2], e.g genus *Maytenus* now includes the species formerly placed in *Gymnosporia.* Similarly, some *Elaeodendron* species are now included in the genus *Reissantia.* As a result, many species have several synonyms. Concerning *Maytenus*, it was suggested that this large and widespread genus needed to be recircumscribed [15].

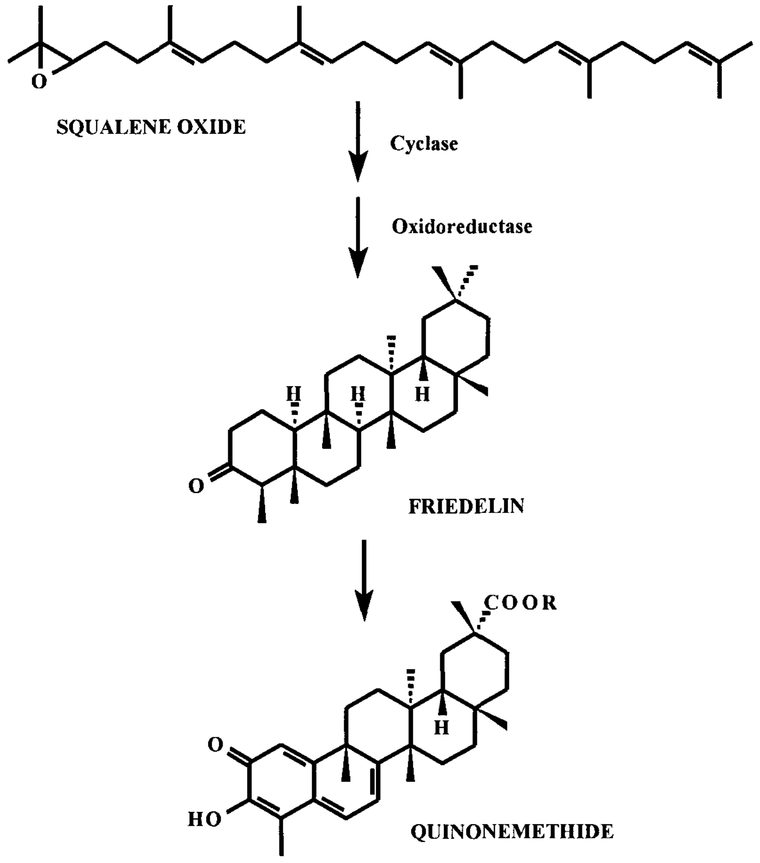
## TRITERPENES

In recent years, an increasing number of reports about a wide variety of biological activities and applications of this type of compounds supported their study, facing the search of potential new drugs. Several articles are found in literature about the use of plants containing triterpenes or the use of triterpenes themselves. Ursolic and oleanolic acids, for example, were reported as effective in protecting against chemically induced liver injury in laboratory animals [16]. Oleanolic acid has been marketed in China as an oral drug for human liver disorders. The physiological function of triterpenes in plants is generally believed to be a chemical defense against pathogens and herbivores. Consequently, there is a great possibility that they could be used against certain pathogens causing human and animal diseases. In the next pages we will discuss the chemistry of recently isolated triterpenes.

## Chemistry

*Friedelanes*

Friedelanes are the most frequently triterpenes isolated from Celastraceae. The co-occurrence of friedelanes and quinonemethides in several species, both in whole plants and cell cultures, has led to propose a biogenetic relationship between these two types of triterpenoids [17,21], Fig. (**1**).

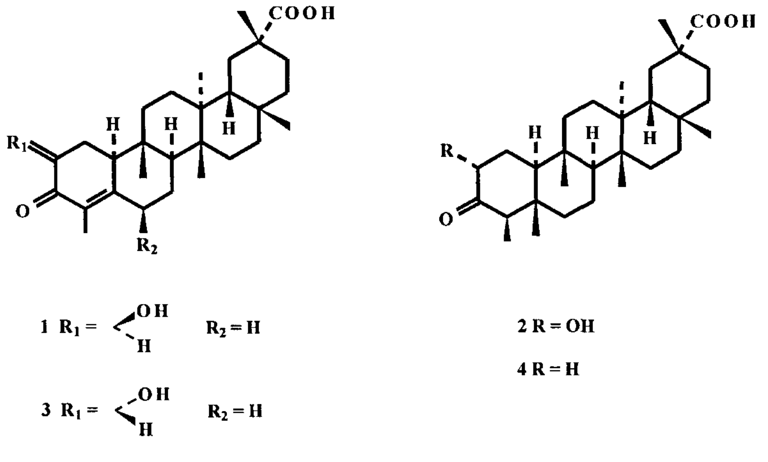



Corsino, F. de Carvalho, Kato, Ribeiro, Oliveira, Araújo, da S. Bolzani, França, Pereira, Furlan (2000)

**Fig. (1).** Conversion of squalene oxide to friedelin and their involvement as the biosynthetic precursor to the quinonemethides in M.aquifolium and S.campestris.

Corsino *et al.* [22] reported that friedelane and quinonemethide biosynthesis is compartmentalized in *Maytenus aquifolium* and *Salacia campestris*, indicating cyclase activity in the leaves but not in the root bark. In order to confirm the possible translocation of friedelin derivatives, from the leaves to root bark, a solution of (±)5-$^{3}$**H**-mevalonolactone was administered to the leaves of *M. aquifolium* seedlings. No labeled friedelin was detected in the root bark but, nevertheless, label quinonemethides were found.

*Tripterygium wilfordii* Hook fil. has been used as anticancer drug and insecticide by the Chinese for centuries [23,24]. The plant has also been used for the treatment of rheumatoid arthritis and spondylitis. An anti-AIDS agents and an anti-HIV principle were isolated from this plant [25]. From this source and his varieties and from *T. hipoglaucum*, Takaishi and coworkers have isolated a series of friedelane triterpenes. From the methanol extract of root barks were isolated a 24-*nor*-**D**:**A**-friedo-oleanane triterpene named wilforic acid B (**1**) and a **D**:**A**-friedo-oleanane named wilforic acid C (**2**) [26]. From spectroscopic data, the former proved to be closely related to regeol B (**3**) [27], isolated from *T. wilfordii* var. *regelii* except for the signals due to C-6, C-7, C-9 and C-10. From these facts and the HREIMS spectrum the structure of wilforic acid B was deduced to be a dehidroxy derivative form of regeol B. The stereochemistry of the hydroxyl group was determined to be β according with the NOESY spectrum and the coupling constants of 2-**H** ($J$ = 14.2 and 6.4 Hz), Fig. (**2**).

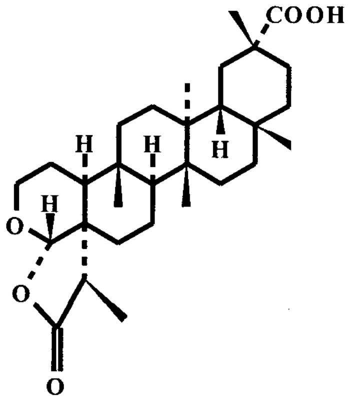



Li, Duan, Kawazoe and Takaishi (1997)

**Fig. (2).** Structures of wilforic acid B (1) and C (2)

The $^{13}C$ spectrum of wilforic acid C was very similar to that from maytenoic acid (**4**) [28], except for the signals belonging to C-1, C-2, C-4 and C-10. The comparison of the spectroscopic data and molecular formula from both compounds led to establish the structure of wilforic acid C having an additional hydroxyl group located at C-2. The relative stereochemistry was determined as α by means of a ROESY experiment and the coupling constant of the geminal proton to the hydroxyl group ($J$ = *ca* 2 Hz).

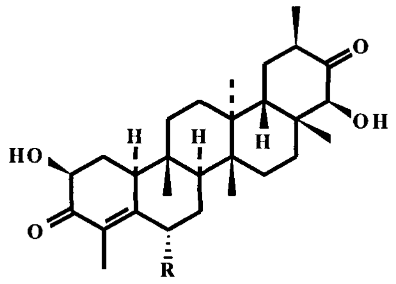
From the outer root bark of *T. hypoglaucum*, celastolide (**5**) was isolated [29]. From the IR and $^{1}H$ and $^{13}C$ NMR spectra, the compound was assumed to be a D:A-friedooleanan type of triterpene. The $^{13}C$ NMR spectrum was very similar to that from salaspermic acid [28] except for the chemical shifts signal assigned to carbons 1, 2, 3, 4, 5, 6, 10 and 23. This suggested that B, C, D, and E rings were the same as in salaspermic acid. From bidimensional NMR experiments and an X-ray diffraction analysis, the structure of celastolide was establish as 2,3-*seco*-2,24-epoxy-D:A-friedooleanane-2,24-olide-29-oic acid, Fig. (**3**).




Duan, Kawazoe, Bando, Kido and Takaishi (1997)

**Fig. (3).** Structure of celastolide (**5** )

From callus cultures of *T. wilfordii*, 24, 29-di*nor* **D**:**A**-friedoolean-4-en-2β,22β-dihydroxy-3,21-dione named triptocalline **A** (**6**) [30] was isolated, Fig. (**4**).



6 R = H Nakano, Oose and Takaishi (1997)

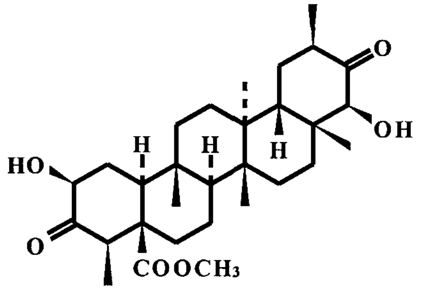
7 R = αOH Yang and Li (2002)

**Fig. (4).** Structures of triptocalline A (6) and 6α-hydroxytriptocalline A (7)

Triptocalline A (**6**) was found to have six methyls and two methines attached to hydroxyl groups. Additionally, the $^{13}C$ NMR spectrum showed signals for a α,β-unsaturated carbonyl group at δ 200.1 and a second carbonyl group at δ 214.0. The spectral data of (**6**) were very similar to those of wilforic acid **B**, except for the signals due to D, and E rings, that were identical to those of 22β-hydroxytingenone [31]. From the evidence discussed the structure of (**6**) is showed in Fig. (**4**).

Recently, the ethanol extract of dried root bark of *T. wilfordii* yielded another triterpene with friedo-oleane skeleton, isolated by Yang *et. al.*[32]. The $^{1}H$ and $^{13}C$-NMR, HMBC, HMQC and NOESY data, and their comparison with those from triptocaline-**A**, allowed to establish the structure of the new compound as 6α-hydroxytriptocalline-**A** (**7**), Fig. (**4**).

Also from *T. wilfordii*, 2β, 22β-dihydroxy-3,21-dioxo-24-carboxyl-29-nor-friedelan methyl ester (**8**) was isolated, Fig. (**5**) [33]. The $^{13}C$ NMR spectrum showed two carbonyl signals assigned to allyciclic ketones, one signal assigned to a carbonyl ester, signals for two oxygenated methines, one methoxy and six methyl carbon signals. It was deduced to be a friedelane-type triterpene, like polpunonic acid, isolated also from the same plant [26]. Further HMBC and NOESY spectral analysis confirmed that compound (**8**) had the structure shown below.

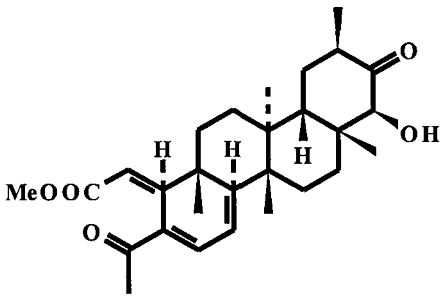


8

Duan, Takaishi, Momota, Ohmoto, Taki, Tori, Takaoka, Jia and Li (1997)

**Fig. (5).** Structure of 2β22β-dihydroxy-3,21-dioxo-24-carboxyl-29-nor-friedelan methyl ester

Triterpenes with $C_{28}$, $C_{29}$ and $C_{30}$ skeletons have been frequently isolated from Celastraceae, but $C_{27}$ type had never been described until 1997. The first compound with such structure, named regelone (**9**) was isolated by Takaishi and coworkers [34] form stem barks of *T. wilfordii* var. *regelii*. The $^{13}$C NMR spectrum showed three carbonyl carbon signals, three double bond carbon signals, seven methyls, five methylenes, three methines and four quaternary carbon signals. The $^{13}$C NMR spectral data for the D- and E-rings were similar to those of 22β-hydroxy-tingenone [31] indicating a ketone group at C-20 and a hydroxyl group at C-21. These data, combined with its high-resolution mass spectrum, supported the proposal of a C-27 triterpene structure with an additional methoxy or carbomethoxy group, according to the molecular formula $C_{28}H_{38}O_5$. In order to confirm the position of the carbomethoxy group, the ketones and other functionalities, the 2D NMR spectra of (**9**) were measured. From those data, the structure was formulated as showed in Fig. (**6**), in which the pentacyclic triterpene skeleton lacks C-3. More than 100 celastrolides have been isolated from Celastraceae family or prepared as derivatives from the congeners [35] but until now the only example of a compound related to regelone is celastrahydride, isolated from *Kokoona zeylanica* by Gamlath and coworkers [36].




9

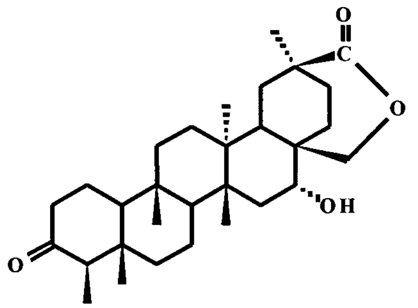
Takaishi, Miyagi, Kawazoe, Nakano, Li and Duan (1997)

**Fig. (6).** Structure of regelone

From the ethanol extract of the dried stems of *Celastrus hindsii* four triterpenes, maytenfolone-A (**10**), celasdin-A (**11**), celasdin-B (**12**) and celasdin-C (**13**) were isolated by Kuo and coworkers Fig. (**7**) [37].

Compound (**10**) showed a characteristic peak at *m/z* 273 in the mass spectrum revealing that the compound was a friedelin derivative [38-40]. The $^{1}H$ and $^{13}C$ RMN data suggested that (**10**) is a lactone derived from friedelin with and additional hydroxyl group. The position of this group was located at C-16 by means of a HMBC experiment and the chemical shifts of carbons C-15, C-16 and C-17 in the $^{13}C$ NMR spectrum. The stereochemistry of the group was determined as $\alpha$ equatorial by means of a NOESY spectrum and from a X-ray diffraction analysis.

Compound (**11**), that was more polar than (**10**), belongs also to the friedelin type skeleton, according with its MS spectrum and the $^{1}H$ and $^{13}C$ DEPT NMR data. The structure of (**11**) was very similar to (**10**), except for the presence of one more methyl group and a hydroxymethylene group instead of the oxidized methylene and the carbonyl group of the lactone. With the aid of 2D NOE and HMBC experiments, the position of the hydroxyl and $CH_2OH$ groups were determined at C-16 and C-20, respectively. These data, together with the MS spectrum confirmed unambiguously the structure of (**11**).

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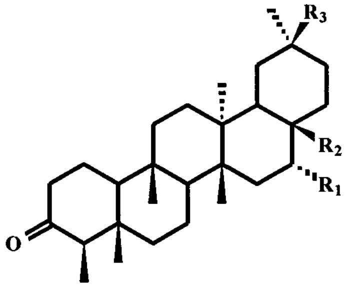
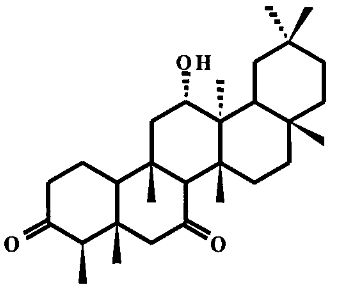



11 $R_1 = OH, R_2 = CH_3$, $R_3 = CH_2OH$
12 $R_1 = OH, R_2 = CH_2OH, R_3 = CH_3$




13

Kuo and Yang (1.997)

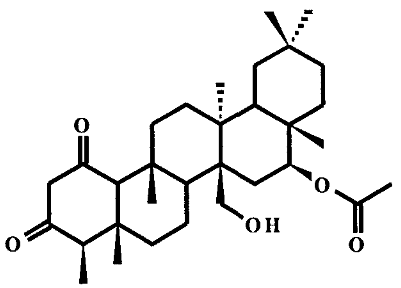
**Fig. (7).** Structures of maytenfolone-A (**10**), celasdin-A (**11**), celasdin-B (**12**) and celasdin-C (**13**)

Compound (**12**) was identified as a triterpene of the friedelin type, with a structure very similar to (**11**), except for the shift of the position of the hydroxymethylene group in (**12**). The position of this group and its stereochemistry was determined by means of HMBC and NOESY experiments, locating it on C-17.

Compound (**13**) showed also a friedelin type skeleton, according to its MS, $^1$H and $^{13}$C DEPT spectra, bearing two carbonyl carbons and one hydroxyl group. With the HMBC spectrum data, the position of the ketone groups were assigned to C-3 and C-7 positions, respectively, as it was observed in putranjivadione [41], isolated from the same plant. The location of the hydroxyl group was assigned at C-12 according with the

long-range correlation between protons of the C-27 methyl group and the carbon bearing the hydroxyl. Other long-range correlation discarded positions C-15 and C-19 that could carried the substituent. Its stereochemistry was established by a NOESY experiment as α, based on the correlations among H-25 and H-26 with H-12.

*Salacia oblonga* Wall. have been extensively used as a remedy for treatment of various diseases in the Ayurvedic system of traditional Indian medicine. From the aqueous methanol extract of the roots of *S. oblonga*, kotalagenin 16-acetate (**14**) was isolated by Matsuda and coworkers [42]. Comparison of the $^{1}H$ and $^{13}C$-NMR data for (**14**) indicated a friedo-oleane type triterpene structure, showing signals for a methylene carrying a hydroxyl group, an acetoxy and two carbonyl moities. The positions of the groups were determined by a HMBC experiment, and the stereochemistry by a NOESY spectrum, that were in agreement with the structure shown below, Fig. (**8**).




**14**

Matsuda, Murakami, Yashiro, Yamahara and Yoshikawa (1999)

**Fig. (8).** Structure of kotalagenin 16-acetate.

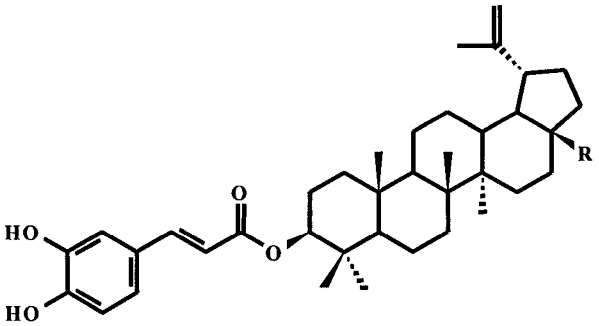
*Lupanes*

Lupane triterpenes are very common in the Celastraceae family. Esters of lupane triterpenes with aromatic acids have been isolated from others sources but in Celastraceae *O*-caffeoyl esters are the most frequently found. C-3 position is the most common compromised with esterification and the stereochemistry of the ester moiety is usually β.

A series of lupane caffeoyl esters have been isolated from *Celastrus stephanotifolius* and *Hippocratea volubilis* by Chen and coworkers [43] and our research group [44].

From *C. stephanotifolius* betulinaldehyde-3β-yl-caffeate (**15**) was isolated. Its structure was determined by spectroscopy. 2D NMR experiments assigned the position of the O-caffeoyl group and the aldehyde at C-3 and C-28, respectively. The stereochemistry of the ester moiety was determined by a NOESY spectrum as β.

From the rootbark of *H. volubilis* lupeol caffeate (**16**) was isolated. Its spectroscopic data were very similar to (**15**) except for the presence of an additional methyl group and the lacking of the aldehyde moiety. The ester group was located at C-3, according to the long-range correlations due to interactions between the geminal proton and the ester carbonyl carbon, and between this proton and C-23 and C-24 methyl groups. Its stereochemistry was determined as β by means of a ROESY experiment




| | R | |
|---|---|---|
| **15** | CHO | Chen, Duan and Takaishi (1999) |
| **16** | $CH_3$ | Alvarenga and Ferro (2000) |

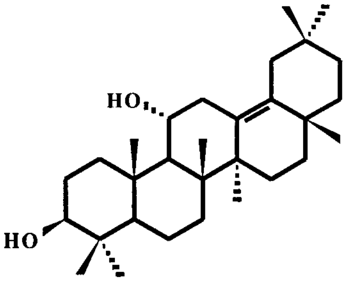
**Fig. (10)**. Structures of betulinaldehyde-3 β-yl-caffeate ( **15**) and lupeol caffeate ( **16**).

*Oleanes*

Oleanes comform the other group of triterpenes found in Celastraceae that- with friedelanes- are the most common skeletons isolated from the family.

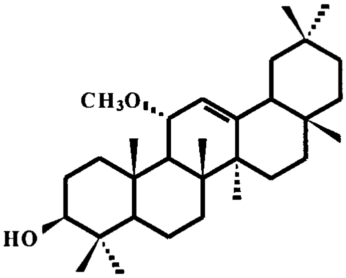
From the dried root outer bark of *T. hypoglaucum* two new triterpenes were isolated [29, 45], Fig. (**11**).

Hypodiol (**17**) showed IR bands of hydroxyl groups. The NMR spectroscopic data showed signals assigned to two carbons bearing secondary hydroxyl groups and a double bond in addition to the signals of eight methyl groups. The HMQC, $^{1}H$-$^{1}H$ COSY and HOHAHA spectra confirmed the position of the hydroxyl groups and the double bond. The hydroxyl groups were assigned at C-3 and C-11 and the double bond was located between C-13 and C-18. The relative stereochemistry of the hydroxyl groups at C-3 and C-11 were determined by means of a NOESY spectrum to be 3β and 11α.




17

Duan, Kawazoe, Bando, Kido and Takaishi (1997)



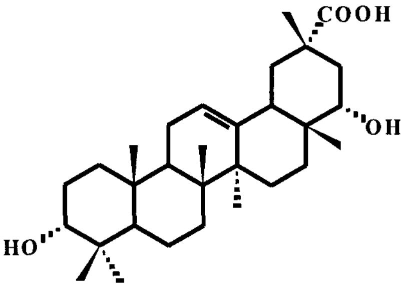

18

Fujita, Duan and Takaishi (2000)

**Fig. (11)** Structures of hypodiol (**17**) and tryptohypol F (**18**)

Triptohypol F (**18**) showed the presence of one double bond, two methine carbons attached to an oxygen function and one methoxy group, according to the $^{13}C$ NMR spectrum. From these data and its $^{1}H$ NMR and MS spectra, it was assumed to be an oleanane-type triterpene. The location of the double bond and the hydroxyl and methoxy groups were determined by a HMBC experiment. The stereochemistry of the hydroxyl and methoxy groups were determined by a NOESY spectrum. According to these data the structure of (**18**) was established as showed above.

From callus cultures of *T. wilfordii*, triptocallic acid D (**19**) was isolated [30]. The molecular formula obtained from a FAB-MS spectrum and the $^{1}H$-NMR spectroscopic data showed that (**19**) had a β-amyrin skeleton. The $^{13}C$ NMR spectrum showed one carboxylic acid, two methines attached to oxygen functions and a double bond. The positions of the groups were determined by $^{1}H$-$^{13}C$ COSY and $^{1}H$-$^{13}C$ long range COSY spectra. The stereochemistry of the hydroxyl groups were determined as α by means of a NOESY experiment and the coupling constants of the geminal protons. According to the above evidence, the structure of triptocallic acid D was shown to be 3α,22α-dihydroxy-olean-12-en-29-oic acid, Fig. (**12**).



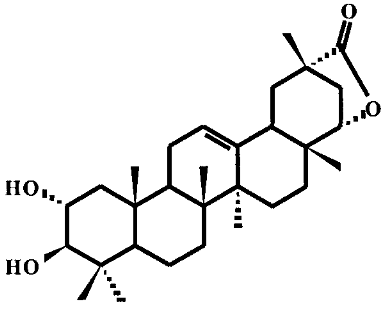

19

Nakano, Oose and Takaishi (1997)

**Fig. (12)** Structure of triptocallic acid D

Also from *T. wilfordii* [33] others six triterpenes have been isolated. 2α,3β-dyhydroxy-olean-12-ene-22,29-lactone (**20**) had an olefinic proton signal, three hydroxyl methine protons and seven tertiary methyl signals based on its $^{1}H$ NMR spectrum. Examination of its $^{13}C$ NMR data showed

30 carbons. The compound was assumed to be a 12-ene oleanane-type triterpene. Based on its HMBC spectrum, the two hydroxyl groups were assigned to C-2 and C-3. Also based on 2D NMR experiments, a C-22, 29-lactone ring was deduced, and this was supported by accounting for the degrees of insaturation suggested by its molecular formula. According of these facts, the structure of (**20**) is showed below, Fig. (**13**).

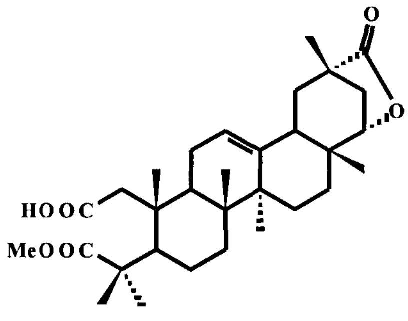



Duan, Takaishi, Momota, Ohmoto, Taki, Tori, Takaoka, Jia and Li (2001)

**Fig. (13).** Structure of 2α,3β-dihydroxy-olean-12-ene-22,29-lactone (**20**).

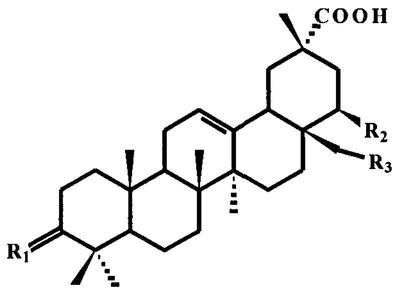
Compound (**21**) showed a $^{13}C$ NMR spectrum very similar to that of (**20**), except for the A-ring. Thus, it was also a 22,29-lactone-oleane-12-ene-type triterpene. According to the degrees of insaturation and the data provided by the HMBC experiment, the A-ring showed to be disunited between C-2 and C-3. Thus, (**21**) was elucidated to be 2,3-*seco*-22,29-lactone-oleane-12-ene-2,3-dioic acid 3-methyl ester, Fig. (**14**).

Compounds (**22**), (**23**) and (**24**) were also 12-ene oleane-type triterpenes based on comparison of their $^{13}C$ NMR spectra with that of (**20**). They differed with regard to the substituted functions at C-3. According to its 2D NMR spectroscopic data, (**22**) was 28-hydroxy-3-oxo-olean-12-en-29-oic acid. In the same way, the coupling constant of H-3 in (**23**) and (**24**) and analysis of the NOESY spectrum suggested that (**23**) and (**24**) were 3β,28-dihydroxy-olean-12-en-29-oic acid and 3α,28-dihydroxy-olean-12-en-29-oic acid respectively, Fig. (**15**).




Duan, Takaishi, Momota, Ohmoto, Taki, Tori, Takaoka, Jia and Li (2001)

**Fig. (14)**. Structure of 2,3-*seco*-22,29-lactone-oleane-12-ene-2,3-dioic acid 3-methyl ester ( **21**).



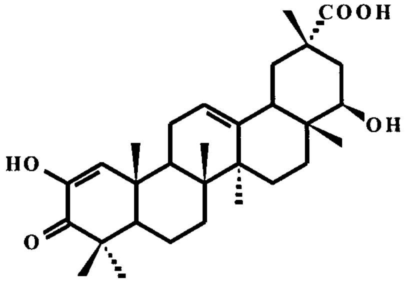

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **22** | O | H | OH |
| **23** | β-OH, α-H | H | OH |
| **24** | α-OH, β-H | H | OH |

Duan, Takaishi, Momota, Ohmoto, Taki, Tori, Takaoka, Jia and Li (2001)

**Fig. (15)**. Structure of compounds ( **22**), (**23**) and (**24**).

Compound (**25**) had a molecular formula of $C_{30}H_{44}O_5$ based on HREIMS. The $^1H$ NMR spectrum showed two olefinic protons and an oxygenated methine. Based on its $^{13}C$ NMR and HMBC spectra and

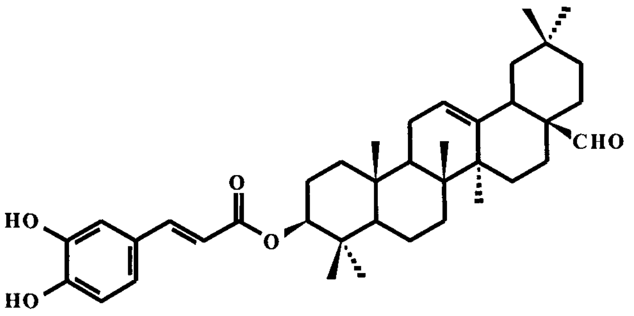
additional literature data [46], (**25**) was determined to be 2,22β-dihydroxy-3-oxo-olean-1,12-dien-29-oic acid, Fig. (**16**).




Duan, Takaishi, Momota, Ohmoto, Taki, Tori, Takaoka, Jia and Li (2001)

**Fig. (16)**. Structure of 2 ,22β-dihydroxy-3-oxo-olean-1,12-dien-29-oic acid ( **25**).

Caffeate esters are found also in the oleane series. From the methanol extract of the stems of *C. stephanotifolius* compound (**26**) was isolated [43], Fig. (**17**). The compound showed a hydroxyl and ketone bands in its IR spectrum. The HREIMS spectrum gave a molecular formula of $C_{39}H_{54}O_5$. From its $^1H$ and $^{13}C$ NMR spectroscopic data, (**26**) showed to have one *O*-caffeoyl group, an aldehyde moiety and that its C-skeleton contained 30 carbons. To confirm the position of the *O*-caffeoyl and aldehyde groups, the 2D NMR spectra were measured. Based on the correlations found, the *O*-caffeoyl group was confirmed to be at C-3, and the aldehyde group was at C-28. The relative stereochemistry of the *O*-caffeoyl group was determined to be 3β according to the NOESY spectrum.

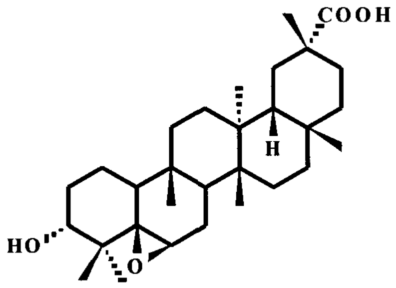



Chen, Duan and Takaishi (1999)

**Fig. (17)**. Structure of compound ( **26**).

*Glutinanes*

From callus cultures of *T. wilfordii*, triptocallic acid C (**27**) was isolated [30]. Its molecular formula was determined as $C_{30}H_{48}O_4$ by HREIMS. The compound showed hydroxyl, carbonyl and epoxide absorptions in its IR spectrum. The position of the functional groups were determined based on the fragments found in its EI-MS spectrum and the 2D NMR spectroscopic data, in addition with the degree of insaturation and the chemical shifts of the carbons bearing the oxygenated functions. The stereochemistry was determined according with its NOESY spectrum. With this evidence, the structure of (**27**) was shown to be 3β-hydroxy-**D:B** friedoolean-5β,6β-epoxy-29-oic acid, Fig. (**18**).

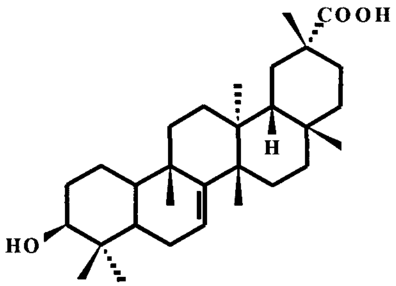



Nakano, Oose and Takaishi (1997)

**Fig. (18).** Structure of triptocallic acid C ( 27).

*Multifloranes*

The only example of this type of skeleton found was triptocallic acid B (**28**), isolated from tissue cultures of *T. wilfordii* by Nakano and coworkers [47]. The compound showed a molecular formula of $C_{30}H_{48}O_3$, according with its HREIMS. Its EI-mass fragmentation pattern were consistent with that of a **D:**C-friedoolean-7-en pentacyclic triterpene [40, 48]. The $^{13}C$ NMR data of (**28**) were similar to those of wilforic acid C [49] for C-15 to C-22 and C-28 to C-30, and swertenol [50] for C-1 to C-14 and C-23 to C-25. Thus, triptocallic acid B was shown to be 3β-hydroxymultiflor-7-en-29-oic acid, Fig. (**19**).



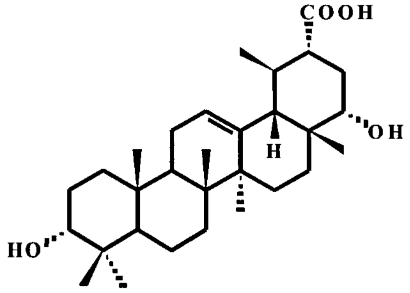

Nakano, Oose, Nasuda, Kamada and Takaishi (1997)

**Fig. (19).** Structure of triptocallic acid B ( **28**).

*Ursanes*

Ursanes are a triterpene skeleton-type commonly isolated from plants of the Celastraceae family.

From callus cultures of *T. wilfordii*, triptocallic acid A was isolated (**29**), [47]. Based on its spectroscopic data (FAB-MS, IR, $^{1}H$, $^{13}C$ and 2D NMR) and of that from its diacetate derivative (obtained from acetylation of (**29**) with acetic anhydride-pyridine), the structure of the compound was determined to be as shown in fig. (**20**).




Nakano, Oose, Nasuda, Kamada and Takaishi (1997)

**Fig. (20).** Structure of triptocallic acid A ( **29**).

Also from *T. wilfordii* (from the water/chloroform extract of the roots), two new ursane triterpenes were isolated by Duan *et al.* [33].

Compound (**30**) showed a ketone, a carboxylic carbon, one double bond and one oxygenated methine in its $^{13}$C NMR spectrum. Its spectroscopic $^{13}$C NMR data were very similar of that of ursolic acid [51]. The position of the functional groups were determined by means of a HMBC experiment and the stereochemistry of H-22 was determined based on its coupling constant, indicating an equatorial configuration. Therefore, (**30**) was elucidated to be 22β-hydroxy-3-oxo-12-ursen-30-oic acid, Fig. (**21**).

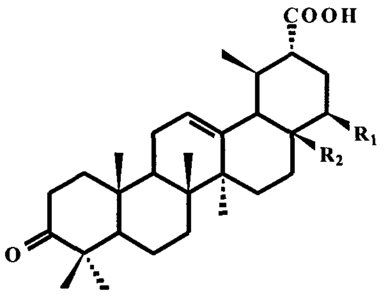
The $^{13}$C NMR spectrum of (**31**) was very similar of that of (**30**), except for one oxygenated methylene in (**31**) and one oxygenated methine in (**30**).Further 2D NMR spectral analysis confirmed that (**31**) was 28-hydroxy-3-oxo-12-ursen-30-oic acid, Fig. (**21**).

From the root bark of *T. hypoglaucum*, triptohypol D (**32**) and triptohypol E (**33**) were isolated [45].

Compound (**32**) showed hydroxy absorption band in its IR spectrum. According with its $^{1}$H and $^{13}$C NMR data, the compound showed a secondary hydroxyl group, a tertiary double bond and an ethoxy group. Based from the carbon number ($C_{30}$) and the presence of two secondary methyl groups, compound (**32**) was assumed to be an ursan-type triterpene. From its 2D NMR spectral data, the hydroxyl and ethoxy groups were assigned to C-3 and C-11, respectively. The relative configuration of the hydroxyl and ethoxy groups were C-3β and 11α, based on a NOESY spectrum. Therefore, the structure of (**32**) was determined as shown, Fig. (**22**).

Compound (**33**) was assigned the molecular formula $C_{31}H_{44}O_2$. Its $^{13}$C NMR spectral data were very similar to those of (**32**) except for one methoxy group signal in the case of (**33**). According with the 2D NMR spectroscopic data, the methoxy group was attributed to C-11, and was assigned C-11α configuration based on its NOESY spectrum, Fig. (**22**).

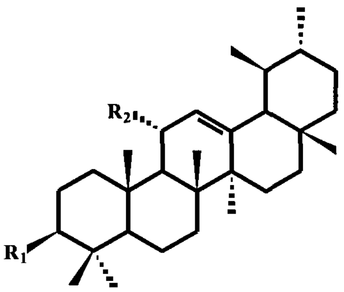
From the MeOH extract of the branches of *Tripterygium doianum* 3β-acetoxy-11-ursen-13α,30-olide was isolated (**34**) [52]. Its IR spectrum showed a lactone carbonyl and a carbonyl band. Based on its $^{1}$H and $^{13}$C NMR spectroscopic data, compound (**34**) was assumed to be an ursene-type triterpene. The compound showed signal for a lactone group, an acetoxy group and a double bond.




| | $R_1$ | $R_2$ |
|---|---|---|
| 30 | OH | Me |
| 31 | H | $CH_2OH$ |

Duan, Takaishi, Momota, Ohmoto, Taki, Tori, Takaoka, Jia and Li (2001)

**Fig. (21).** Structures of compounds ( **30**) and (**31**).

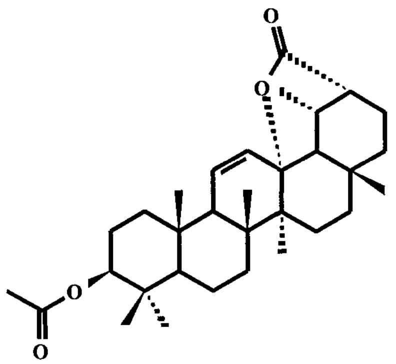



| | $R_1$ | $R_2$ |
|---|---|---|
| 32 | OH | $OCH_2CH_3$ |
| 33 | OH | $OCH_3$ |

Fujita, Duan and Takaishi (2000)

**Fig. (22).** Structures of triptohypol D ( **32**) and E (**33**)

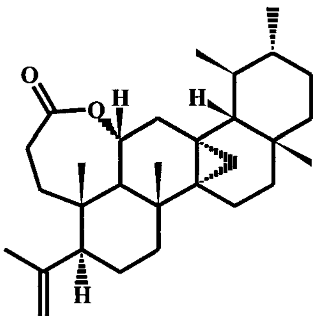
The location of the substituents was determined from a HMBC experiment. According to this data, the acetoxy group was located at C-3, the double bond between C-11 and C-12 and the lactone ring between C-13 and C-20. The relative stereochemistry of the acetoxyl were determined based on the coupling constant of the geminal proton and the data from the NOESY spectrum. The relative stereochemistry of the lactone group was determined also based from a NOESY experiment. According to those facts, the structure of (**34**) is showed below, Fig. (**23**).




Tanaka, Duan, Takaishi, Kawazoe and Goto (2002)

**Fig. (23).** Structure of 3 β-acetoxy-11-ursen-13 α,30-olide (**34**)

From the stem bark of *Glyptopetalum sclerocarpum*, glyptopetolide (**35**) was isolated [53]. The compound showed signals of protons of a cyclopropane ring and a isopropenyl group in its $^{1}$H NMR spectrum. A lactone moiety was also determined from the $^{13}$C NMR spectral data. Compound (**35**) proved to be a 3,4-*seco* ursane triterpene, according from its HMBC spectrum. Also based on the HMBC experiment, the location of the lactone group, as well as the cyclopropane and the isopropenyl groups were determined. The stereochemistry was based on a NOESY experiment. Therefore, the structure of (**35**) was elucidated as 3,4-*seco*-14α,27-cyclo-urs(23)-en-3,11α-olide, a very singular structure, considering the 3,4-*seco* nature of ring A and the presence of a cyclopropane ring in the C/D ring junction, Fig. (**24**).




Sotanaphun, Suttisri, Lipipun and Bavovada (2000)

**Fig. (24).** Structure of glyptopetolide (**35**).

*Dammaranes and related compounds*

This triterpene skeleton-type is not very common in Celastraceae. Not many examples were found in the literature covered by this review, and most of them were reported for a *Maytenus* species.

From the stem bark exudates of *Maytenus macrocarpa*, nine dammarane triterpenes were isolated by Chávez *et al.*[54].

Compound (**36**) was identical of the compound previously isolated from *Rapanea umbellate* (Umbeliferae) 24-(*E*)-3-oxo-dammara-20,24-dien-26-al [55].

Compound (**37**) showed NMR spectral data very similar to those of (**36**). The main differences between the two compounds were the chemical shifts of the signals from the proton of the aldehyde group in the $^{1}$H-NMR spectrum and those attributable to carbons C-24, C-25, C-26 and C-27 in its $^{13}$C NMR spectrum. Based on these facts and NOEDIF experiments, (**37**) proved to be the *Z*-isomer of (**36**), Fig. (**25**).

Compound (**38**) showed hydroxyl and carbonyl groups in its IR spectrum. Characteristic in its $^{1}$H-NMR spectrum was the presence of a signal of a methylene group joined to an oxygenated function. The compound was acetylated with $Ac_2O$/Py and transformed to (**36**) by oxidation with $PCC/CH_2Cl_2$. Its structure was established as 24-(*E*)-3-oxo-dammara-20,24-dien-26-ol, Fig. (**25**).

Compounds (**39**) and (**40**), Fig.(**25**) differs from (**36**) in the multiplicity of the signal from H-24 and the presence of a proton geminal to an

hydroxyl group. The location of this group was determined to be at C-23 to both compounds, based on the data provided by a HMBC experiment. The stereochemistry of the double bond between C-24 and C-25 was confirmed as *E* according with a ROESY experiment. All data pointed out that (**39**) and (**40**) were epimeric compounds at C-23. Determination of absolute configuration of the compounds was not possible due to the small amount isolated. Attempts to synthesize the compounds from (**36**) were unsuccessful.

Compound (**41**), Fig. (**25**) showed spectroscopic data very similar of those of (**36**). The main difference was the presence of an additional hydroxyl group that was located at C-6 by means of a HMBC experiment. Its stereochemistry was established by published data [56,57]. The compound did not form the correspondent acetate, probably due to steric interactions at the reaction site.

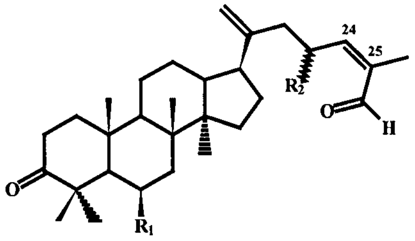
Compound (**42**) was very similar with (**41**) except for the presence of a primary hydroxyl group instead of the aldehyde moiety at C-26. According with this assumption, (**42**) was transformed to (**41**) with PCC/$CH_2Cl_2$. Its structure is shown in Fig. (**25**).

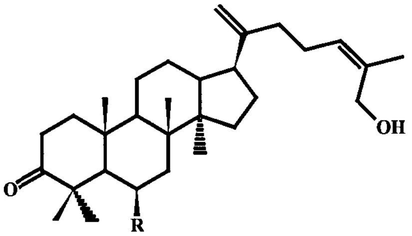
Compound (**43**), Fig.(**25**) showed the presence of 29 carbon atoms and the dammarane nucleus. The main difference was at the lateral chain. With the aid of its HMBC spectrum, the structure of (**43**) was established as 23-(*Z*)-3,25-dioxo-25-nor-dammara-20,24-diene.

Compound (**44**) showed –as principal differences with compounds previously described-and additional double bond and a carboxyl carbon. One secondary hydroxyl group was also found. With the aid of a HMBC experiment, the structure of (**44**) was confirming as a homo-dammarane, Fig. (**25**).

From the MeOH extract of the branches of *T. doianum* two triterpenes were isolated by Tanaka *et al.* [52].

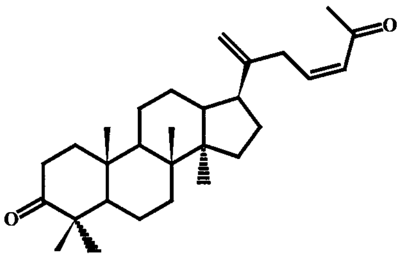
Compound (**45**) showed a molecular formula of $C_{30}H_{49}O_2Cl$ based on its HREIMS. Its spectral $^{13}C$ NMR data were very similar to those of 24*S*,25-dihydroxytirucall-7-en-3-one isolated from *Owenia cepiodora* [58]. The compound showed a ketone group, one hydroxyl group and a chlorine atom according with its spectroscopic data. The location of the groups was determined based on a HMBC spectrum. With these data, the structure of (**45**) was determined as 25-chloro-24-hydroxytirucall-7-en-3-one, Fig. (**26**).

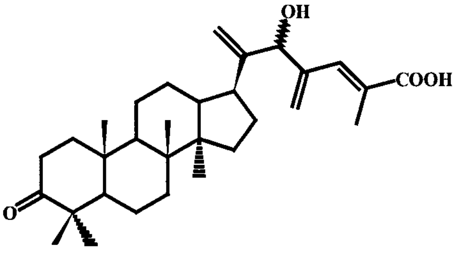



| | | | |
|---|---|---|---|
| 36 | 24-*E* | $R_1 = H$ | $R_2 = H$ |
| 37 | 24-*Z* | $R_1 = H$ | $R_2 = H$ |
| 39 | 24-*E* | $R_1 = H$ | $R_2 = OH$ |
| 40 | 24-*E* | $R_1 = H$ | $R_2 = OH$ |
| 41 | 24-*E* | $R_1 = OH$ | $R_2 = H$ |

| | |
|---|---|
| 38 | R = H |
| 42 | R = OH |



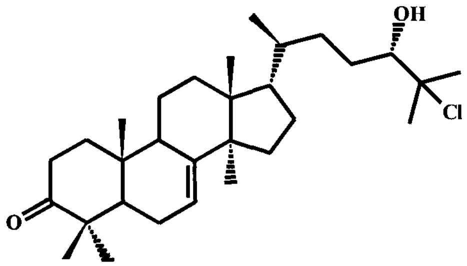

43

44

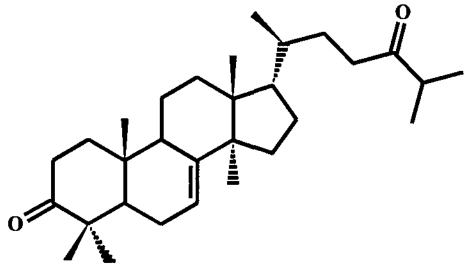
Chávez, Estévez-Braun, Ravelo and González (1997)

**Fig. (25).** Dammarane triterpenes from *Maytenus macrocarpa*

Compound (**46**) proved to be very similar to (**45**), except for the chemical shifts of carbons C-22-27. Also, the lack of the hydroxyl group and the chlorine atom was noted. Instead, a second ketone group was found. The location of the two ketone groups were determined based on the data provided by a HMBC experiment. Therefore, the structure of (**46**) was determined to be 3,24-dioxotirucall-7-en, Fig. (**26**).



45



46

Tanaka, Duan, Takaishi, Kawazoe and Goto (2002)

**Fig. (26).** Structure of compounds ( **45** ) and ( **46** ).

## *Other related compounds*

Among the compounds related to triterpene recently isolated from the Celastraceae family cytotoxic cardenolides and a possible Diels-Alder adduct have been reported in the literature by Ankli *et al.* [59] and our research group [60].

From the roots of *Crossopetalum gaumeri* Ankli and coworkers isolated four highly cytotoxic cardenolides. The methanol extract was fractionated using cytotoxicity against a KB cell line. The compounds showed characteristic signals for a butenolactone ring in its $^{1}$H-NMR

spectrum. From these data and its $^{13}$C-NMR and FAB-MS spectra a cardenolide-type structure was deduced for the compounds.

Compound (**47**), Fig. (**27**) showed signals for an aldehyde group and a β-linked deoxyhexose in its $^{1}$H-NMR spectrum. The location of the aldehyde moiety was confirmed by 2D NMR spectroscopy, as well as the identity of the deoxyhexose, that was identify as β-6-deoxygulose, which was confirmed by TLC after hydrolysis of (**47**) and comparison with authentic β-6-deoxygulose. The aglycon was identified as securigenin by means of 2D NMR spectroscopy and the literature data [61,62].

Compound (**48**), Fig (**27**) showed signals for a hydroxymethyl group instead of the signals for the aldehyde group in its $^{1}$H-NMR spectrum. By means of MS and NMR analysis, the genin was identified as 19-hydroxysarmentogenin [63]. The sugar was identified as β-6-deoxygulose as described for compound (**47**).

Compound (**49**) Fig. (**27**) showed two sugar moieties and a C-19 methyl group, according with its 1D NMR data. The aglycon was identified as sarmentogenin [64]. NMR spectroscopy and TLC determined the identity of the sugars after hydrolysis of (**49**) and comparison with authentic compounds.

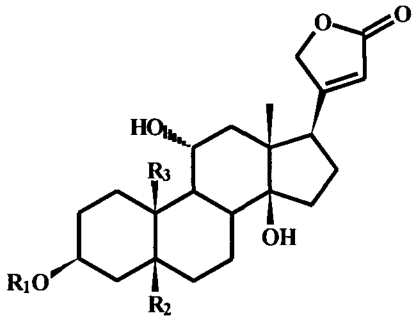
The NMR spectral data of the aglycon of compound (**50**) were very similar of that of compound (**47**) and was therefore identified as securigenin. The nature of the sugars was identical of those of compound (**49**), and the structure of (**50**) is shown in Fig. (**27**).

From the root bark of *H. volubilis*, volubilide (**51**) was isolated. This unusual compound has a structure based on two monomer units from abietane and lupane di/triterpene skeletons. From its HREIMS, $^{1}$H, $^{13}$C-NMR and X-ray data the molecular formula $C_{50}H_{74}O_5$ was obtained for (**51**). From the $^{13}$C-NMR data and 2D NMR experiments, the C-30 unit was identified as a lupane derivative with a 30-21α lactone group, related to ochraceolide A [65].

The data mentioned above pointed out that the C-20-C-29 bond was involved in the linkage with the diterpene unit. This diterpene has never been isolated as a natural product, but was found as a monomer in the dimeric compound maytenone [66].

Compound (**51**) appeared to come from a Diels-Alder reaction between a diterpene diene and ochraceolide A as a dienophile. The possibility of the presence of enzymatic system with hetero or Diels-Alderase activity in Celastraceae was suggested once again, based on the structure of (**51**)

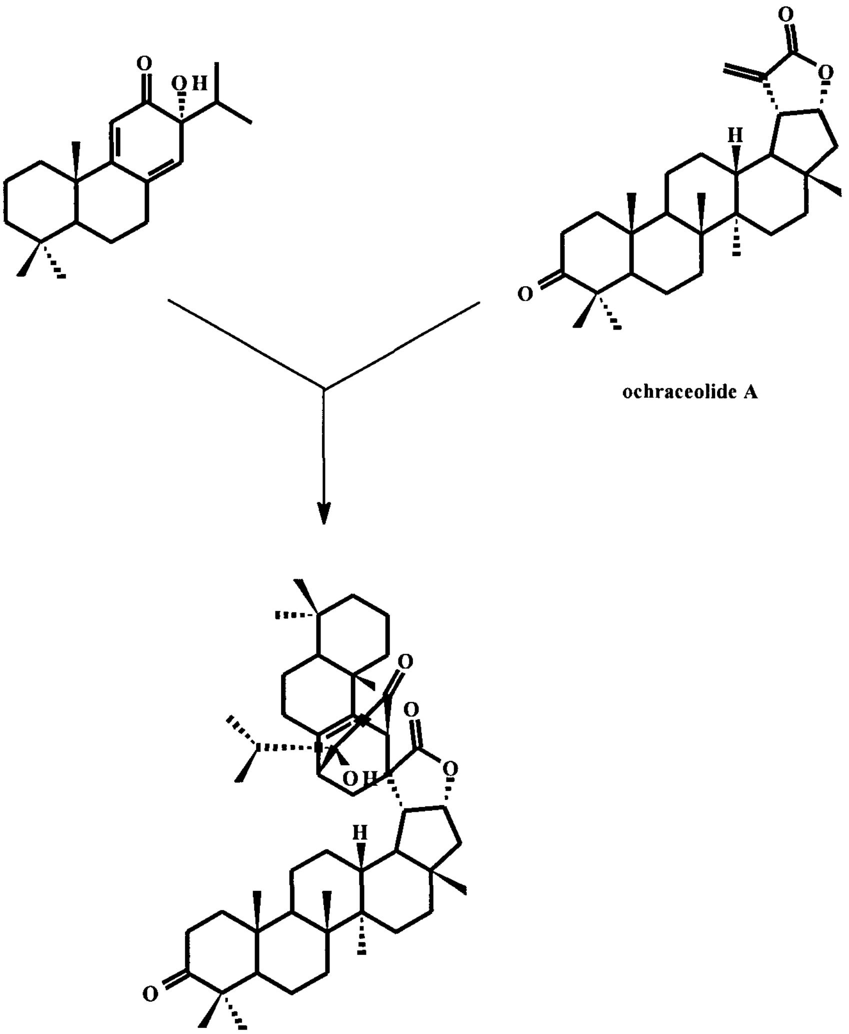
and others secondary metabolites isolated from the family [67]. The structure of (**51**) and its probable mechanism of formation is showed in Fig. (**28**).




| | | | |
|---|---|---|---|
| 47 | β-6-deoxygulosyl | H | CHO |
| 48 | β-6-deoxygulosyl | H | $CH_2OH$ |
| 49 | α-allosyl-(1→4)-β-6-deoxyallosyl | H | $CH_3$ |
| 50 | α-allosyl-(1→4)-β-6-deoxyallosyl | H | CHO |

Ankli, Heilmann, Heinrich and Sticher (2000)

**Fig. (27).** Cytotoxic cardenolides from *Crossopetalum gaumeri*



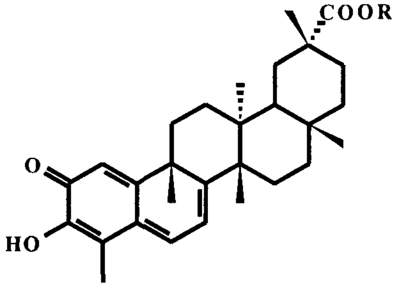

Alvarenga, Ferro, Ravelo, Kennedy, Maestro and González (2000)

**Fig. (28).** Structure and proposed mechanism of volubilide formation (**51**).

# TRITERPENOID QUINONEMETHIDES AND RELATED COMPOUNDS

Triterpenoid quinonemethides or celastroloids, as defined by Brüning and Wagner in a classical review on Celastraceae [2], are compounds with D:A-*freido-nor*-oleanane skeleton characterized by a particular oxygenation pattern and variable type of insaturation. These compounds, which regularly appear along with D:A-*friedo-nor*-oleananes and lupanes, are characteristic secondary metabolites found in the species of this family. At the present time, more than 90 structures of these commonly red-orange pigments, mostly present in the outer root bark of Celastraceae species, were described. In order to have a complete view of the chemistry, biosynthesis and biological activity and these compounds, the comprehensive work of Gunatilaka [35] covers the knowledge about this type of terpenoids up to the middle of 1994. Other review from González *et al.*, covers literature from 1994 to mid 1.997 [68].

Typical triterpenoid quinonemethides are 5-ring triterpenes bearing a ketone at C-2 and an hydroxyl group at C-3. An unsaturated system involves rings A and B. This conjugated system can hold an extra carbonyl group in ring B and the extension of the conjugations could involve either rings C or D. Besides that, a number of compounds with an diphenol system in ring A were also described. The older structures described, celastrol (**52**) and pristimerin (**53**) were isolated from *T. wilfordii* [69] and *Celastrus scandens* [70], and *Pristimeria indica*, respectively [71], Fig. (**29**).



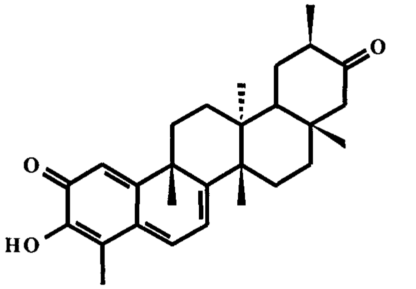

R = H Celastrol (52)

R = $CH_3$ Pristimerin (53)

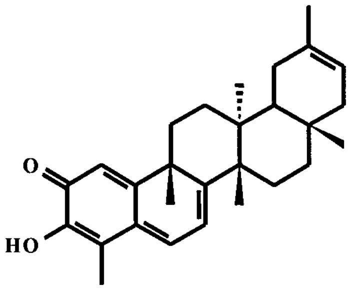
**Fig. (29).** Structures of celastrol and pristimerin

## Compounds with non-extended conjugation

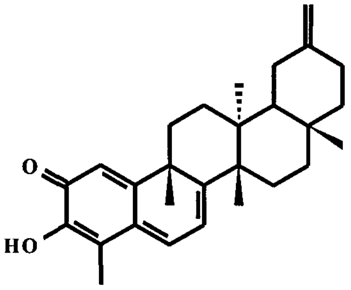
A number of quinonemethides with structures related to pristimerin, tingenone (**54**) iguesterine (**55**) and isoiguesterine (**56**) had been described in these years. All of them are characterized by a conjugated system involving rings A and B, which is named non-extended conjugation, Fig. (**30**).



**54**



**55**

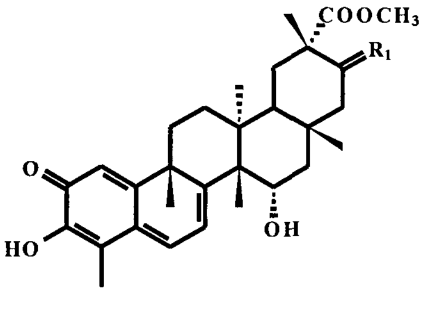


**56**

**Fig. (30).** Structures of tingenone (**54**), iguesterine (**55**) and isoiguesterine (**56**)

From two *Maytenus* species collected in Paraguay, two new pristimerin-related compounds were isolated, 15-α-hydroxypristimerin (**57**), from *M. scutioides* [72], and 15-α-hydroxy-21-keto-pristimerin (**58**) from *M. catingarum* [73], the last one isolated in our laboratory. The structure of compound (**57**) was elucidated by spectroscopic means, including MS, $^{1}H$ and $^{13}C$ – NMR, and $^{1}H$ - $^{13}C$ heteronuclear

correlation. These data showed a pattern close-related to pristimerin, except for a signal assigned to a proton geminal to an additional hydroxyl group, which was located on C-15 based on HMBC experiments. The stereochemistry was established as α-axial based on an ROESY experiment. Additionaly, the treatment of (**57**) with thionyl chloride afforded netzahualcoyene (**59**), a 14(15)-ene-quinonemethide triterpenoid, previously isolated from *M. horrida* [74]. The identification of (**57**) is a key step in the proposal for a biosynthetic pathway of quinonemethides with extended conjugation. The compound (**58**), $C_{30}H_{38}O_6$, also showed spectroscopic data resembling those from pristimerin (**53**). The $^{13}$C-NMR spectrum showed an extra carbonyl group and a carbon bearing one hydroxyl group, which were located on C-20 and C-15, respectively, based on $^{1}$H -$^{13}$C NMR 2D experiments. The stereochemistry of the proton at C-15 was determined as β based on measurements of H-H coupling constants and molecular mechanics calculation, Fig. (**31**).



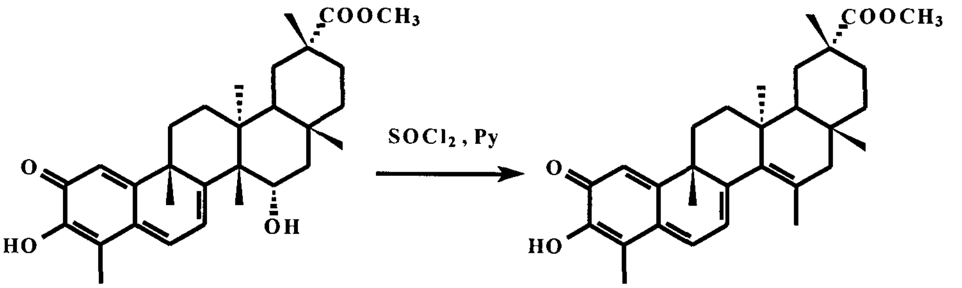

**R$_1$ = αH, βH  15α-hydroxypristimerin (57)**

**R$_1$ = O  15α-hydroxy-21-keto-pristimerin (58)**

(**57**) González, Alvarenga, Bazzocchi, Ravelo and Moujir (1998).

(**58**) Alvarenga, Velázquez, Gómez, Canela, Bazzocchi and Ferro (1999).

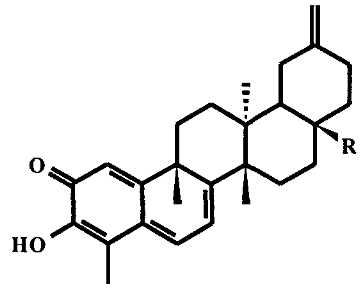
**Fig. (31).** Structures of compounds (57) and (58).




57 59

Fig. (32). Obtention of netzahualcoyene (59) from compound (57)

The first quinonemethide structure with iguesterine (**55**) skeleton was isolated by González and coworkers from *Maytenus canariensis* (*Catha cassinoides*) [75], and its isomer bearing an *exo*-methylene group – isoiguesterine (**56**) was isolated by Sneden from *Salacia madagascariensis* [76]. Related to this scarce type of skeleton, three new structures were isolated from the roots of *Salacia kraussii* [77], all of them exhibiting the characteristic spectroscopic profile of celstroloids. The structures were elucidated by spectroscopic means (COSY, DEPT, $^{1}H$-$^{13}C$-HETCOR and long-range $^{1}H$-$^{13}C$-HETCOR). The three structures showed additional 2-H signals in $^{1}H$-NMR at $\delta$ 4.61-4.64 ppm, which, besides data from $^{13}C$- NMR spectra and HETCOR experiments, allowed the authors to recognize the isoiguesterine skeleton. As a differential feature, these compounds exhibited one less aliphatic methyl group, and the molecular formulas $C_{28}H_{36}O_3$, $C_{28}H_{34}O_3$, $C_{28}H_{36}O_4$, indicating different grades of oxidation. The missing C-28 methyl group was replaced by a primary methyl group in 28-hydroxyisoiguesterine (**60**), and aldehyde in 28-*nor*-isoiguesterine-17-carbaldehyde (**61**), and by a methyl ester moiety in 17-(methoxycarbonyl)-28-*nor*-isoiguesterine (**62**), Fig. (**33**).



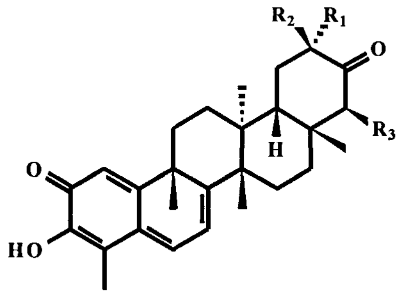
| | R |
|---|---|
| 60 | $CH_2OH$ |
| 61 | CHO |
| 62 | $COOCH_3$ |

Neves, Raz and Sèquin (1998)

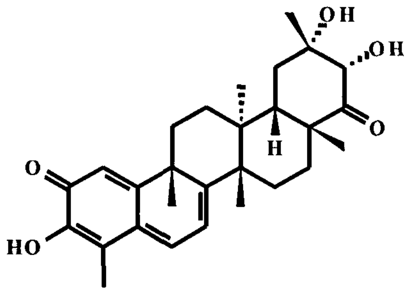
**Fig. (33).** Structures of compounds (**60**), (**61**) and (**62**).

Tingenone (**54**) is with pristimerin the celastroloid more frequently reported, since its isolation from *Euonymus tingens* by Brown *et al.* [78]. Its structure exhibits the characteristic loss of a methyl group of the E ring, which regularly supports and carbonyl group and other oxygen in the congener compounds. An important number of new compounds with this type of skeleton were isolated by Sotanaphun *et al.* [79] from the stem bark of *G. sclerocarpum*. Along with the known compounds tingenone, 22β-hydroxytingenone and 20-hydroxy-22-*epi*-tingenone, was isolated 20-hydroxytingenone (**63**) as a natural product, and its structure was elucidated by $^{1}H$ and $^{13}C$- NMR spectral data. The configuration at C-20 was determined by NOE experiments showing this effect between methyl groups C-28 and C-30 and between the methyl group at C-27 and the hydroxyl at C-20. Three compounds showed the formula $C_{28}H_{36}O_5$, revealing the presence of an extra hydroxyl group. Two of them showed a set of spectroscopic data closely related to compound (**63**), except by the replacement of methylene at C-22 by a downfield methine carbon. This feature suggested the occurrence of a pair of 20,22-dihydroixytingenone derivatives. The relative stereochemistry of the two compounds was

determined by NOE and NOESY experiments, showing the same configuration al C-22, but being epimer at C-20. These compounds were named 20,22β-dyhydroxytingenone (**64**) and 20,22β-dyhydroxy-20-*epi*-tingenone (**65**), respectively. Also related to the structure of these compounds, was isolated 20,21α-dyhydroxy-22-oxo-21desoxotingenone (**66**), which structure was elucidated based on NMR experiments showing the shift of carbonyl group from C-21 to C-22. The relative orientation of H-21 and the methyl group on C-20 was described as β based on NOE experiments Fig.(**34**).




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 63 | OH | $CH_3$ | H |
| 64 | OH | $CH_3$ | OH |
| 65 | $CH_3$ | OH | OH |



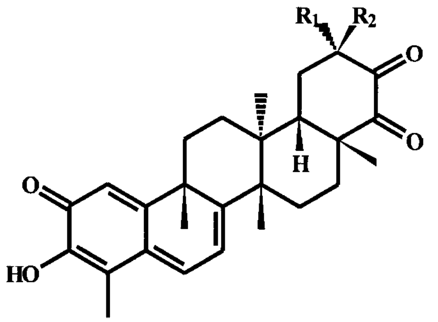

66

Sotanaphun, Suttisri, Lippipun and Bavovada (1998)

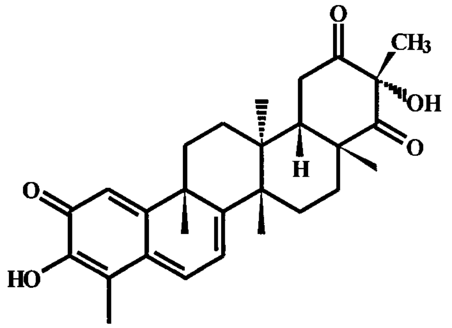
**Fig. (32).** Structures of compounds ( 63), (64), (65) and (66)-

Following the description of the new products isolated from *G. sclerocarpum* by Sotanaphun *et al.,* three of them showing the same molecular formula, $C_{28}H_{34}O_5$, exhibit an extra carbonyl group located on ring E. The structure of these compound was determined by spectroscopic methods, mainly MS, $^{13}$C-NMR, HMBC and NOE. For all of them (compounds **67**, **68**, **69**) a carbonyl group was assigned to the C-22 position, based on the connectivity displayed with H-18 and the C-28 methyl group. The remaining carbonyl group was assigned to the position 21 for compounds (**67**) and (**68**) due to the long range HMBC correlation exhibited with the proton H-19α. For the compound (**69**) such correlation was established with the proton H-18, assuming that the carbonyl group is located on C-20. Another characteristic feature of these three compounds was the occurrence of a quaternary carbon bearing both a methyl and an hydroxyl group, which was assigned to the position C-20 for the compounds (**67**) and (**68**), and to the position C-21 – between two carbonyl groups – for the compound (**69**), respectively, according to three-bond correlations exhibited by the mentioned quaternary carbon and the nearby protons (H-18, H-19α). The NOE experiments let the authors conclude that the difference in the structure of (**67**) and (**68**) was limited to the stereochemistry at C-20, being the epimers 20-hidroxy-22-oxotingenone (**67**) and 20-hydroxy-22-oxo-20-*epi*-tingenone (**68**). The same type of observation applied to the compound (**69**) allowed to establish the configuration of C-21 as α-OH/β-$CH_3$, and therefore the structure of this novel celastroloid as 21α-hydroxy-20,22-dioxo-30(20→21)*abeo*-21-desoxotingenone, Fig. (**35**).

Two compounds, (**70**) and (**71**) from the same source, exhibiting as molecular formulas $C_{28}H_{36}O_5$ and $C_{27}H_{34}O_4$, respectively, showed spectroscopic characteristics similar to other celastroloids, but the occurrence of an extra methyl group, two methylene and two quaternary carbons in DEPT spectra of (**70**) suggested the presence of *seco* character associated to ring E. Based on the correlation observed between H-19 and an acetyl moiety, that of methylene with C-17, and the definition of the quaternary carbon as a terminal carbonyl group, the structure of (**71**) was established as 20-oxo-20,21-*seco*-tingen-21-oic acid, Fig. (**36**).



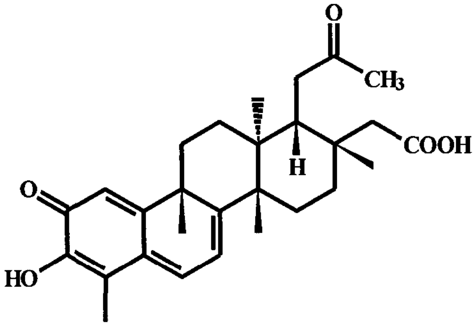

| | $R_1$ | $R_2$ |
|---|---|---|
| 67 | OH | $CH_3$ |
| 68 | $CH_3$ | OH |



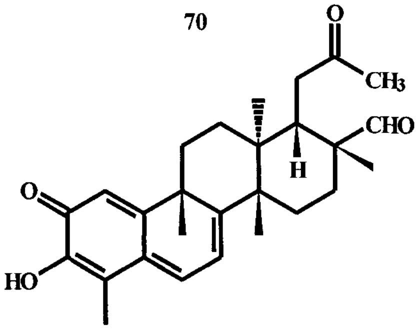

69

**Fig. (35).** Structures of compounds (67), (68) and (69)

The other related compound showed similar characteristics than (**70**) and was evident the loss of a quaternary carbon, different from that attached to C-18. The presence of a singlet at δ 9,71 ppm in $^1$H-NMR and the assignation of a formyl group at C-17, based on the 3-bond correlations with H-16β, H-18 and the methyl group 28, resulted in the description of (**71**) as 20-oxo-21-nor-20,21-*seco*-tingen-22-al, Fig. (**36**).




70




71

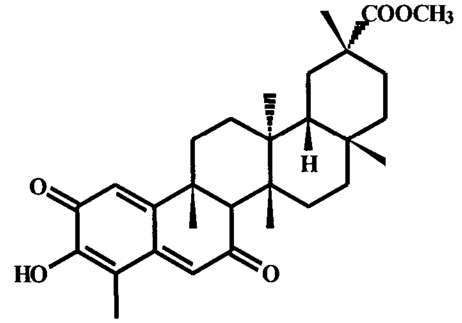
**Fig. (36).** Structures of compounds (**70**) and (**71**)

## Compounds with carbonyl group in B ring

The first structure of a celastroloid described with this type of modification was the 7-oxoquinonemethide dispermoquinone (**72**), isolated from *M. dispermus* [80], Fig. (**37**).

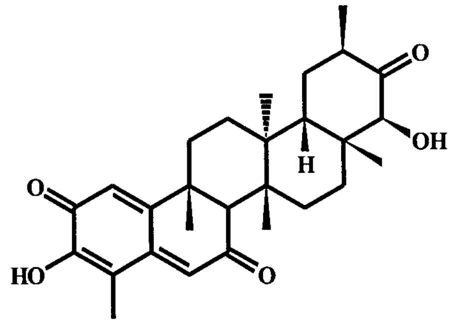
Bearing a 7-oxoquinonemethide structure, but with a skeleton related to tingenone insted of pristimerin, as exhibited by dispermoquinone, a new celastroloid (**73**) of this uncommon type was isolated from the roots of *M. amazonica* by Chávez *et al.* [81]. The structure was elucidated by spectroscopic evidences, exhibiting a molecular formula $C_{28}H_{32}O_5$, and characteristic features of 7-oxo-quinonemethides in the $^1H$ NMR spectrum as the signals of H-1 and H-6 as non-interchangeable with $D_2O$

singlets at δ 6,39 and 6,45 ppm. These data plus other spectroscopic evidence ([13]C NMR, DEPT, HMQC, HMBC) allowed the authors to establish the structure of **(73)** as 7,8-dihydro-7-oxo-22β-hydroxytingenone, Fig. **(38)**.




Martin (1973)

**Fig. (37).** Structure of dispermoquinone (72)



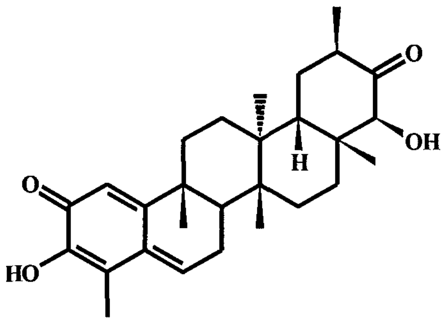

Chávez, Valdivia, Estévez-Braun and Ravelo (1998)

**Fig. (38).** Structure of compound (73)

## Compounds with modified conjugation

A number of celastroloids with extended conjugation has been described [35]. But compounds lacking an insaturation on ring B had not been reported. A compound of this type (**74**) was also isolated from the roots of *M. amazonica* by Chávez *et al.* [81] as an amorphous white solid, unlike the yellow to red colors exhibited by the rest of the quinonemethide triterpenoids.

The structure of the compound $C_{28}H_{38}O_4$ was elucidated by spectroscopy. The $^1$H-NMR spectrum was quite similar to that from 22-β-hydroxytingenone, but showing a triplet at δ7.30 ppm (1H, J= 6.7 Hz). This signal showed NOE effect with the methyl group on C-4, and was evidenced a coupling by $^1$H-$^1$H-COSY experiment with a multiplet at δ 2.51 ppm. These data plus the results from HMQC and HMBC experiments let to establish the structure as 7,8-dihydro-22β-hydroxytingenone (**74**). The authors suggested that this compound could be a key precursor in the biogenesis of compound (**73**) by oxidation.




Chávez, Valdivia, Estévez-Braun and Ravelo (1998)

**Fig. (39).** Structure of compound (**74**)

## Phenolic triterpenoids

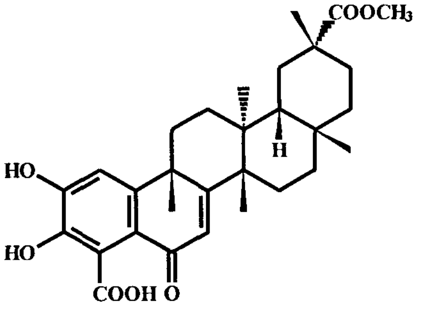
The first natural celastroloid-related structure described bearing a phenolic system in ring A was zeylasterone (**75**), isolated from *Kokoona zeylanica* [82]. That compound was characterized as a 6-oxo-diphenol derivative of pristimerin (**53**).

From *T. wilfordii* var. *regelii* stem bark two new phenolic structures were isolated by Takaishi *et al.* [27]. One of these structures, named regeol A (**76**), is related to tingenone, and the other, regeol C (**77**), is a 6-oxo-pristimerin derivative. The structure of regeol A (**76**) was determined by spectroscopic evidence. The aromatic character was recognized by UV absorption maxima at 240 and 286 nm, and by $^{13}$C-NMR data. $^{1}$H-RMN revealed the presence of 6 methyl groups, 5 of them as singlets (δ 0.85 – 2,01 ppm) and one doublet (δ 1.05 ppm, *J*= 5.9 Hz), a methine attached to oxygen (δ 4.55ppm, *J*= 3.4 Hz) and one methine proton attached to an aromatic ring (δ 6.65 ppm). Its MS spectrum showed a peak at *m/z* 440 that agrees with the formula $C_{28}H_{40}O_4$. The comparison of $^{13}$C-NMR data with those from other celstroloids derivatives showed a similar structure in rings C, D and E as observed in the previously described 22β-hydroxytingenone (tingenin B) [31]. The $^{1}$H-$^{13}$C long range correlation spectrum allowed to locate the aromatic system in ring A. The acetylation of compound (**76**) yielded a triacetate, supporting the structure proposed for regeol A or 22β-hydroxytingenol.

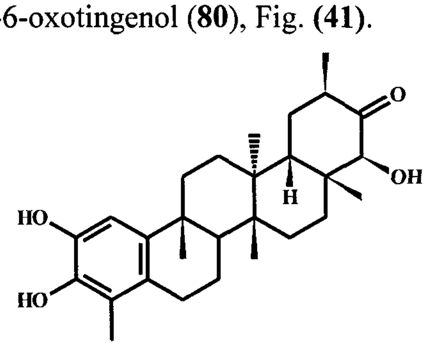
The structure of regeol C (**77**), was also determined by spectroscopic means, showing similar characteristics than regeol A at IR and UV spectra. $^{13}$C-NMR and $^{1}$H-RMN data revealed the presence of 6 methyl groups, all of them singlets (δ 1.12 – 3.18 ppm) and a methine proton attached to an aromatic ring (δ 7.25 ppm). These data and the formula $C_{29}H_{38}O_6$, obtained by FAB-MS agrees with a structure of a pristimerin-derived phenol. NMR spectra of compound (**77**) was quite similar to that of the known compound 23-*nor*-6-oxo-demethylpristimerinol (wilforol A) (**78**), also isolated from this plant [83]. $^{1}$H-RMN spectrum of (**77**) just differs in the signals assigned to protons on positions C-7 and C-15, and $^{13}$C-RMN data showed differences in the signals assigned to positions C-6, C-7, C8 and C-24. Based on these evidences the authors determined the structure of (**77**) as regeol C, that could be named 23-*nor*-7-hydroxy-6-oxo-demethylpristimerinol, Fig. (**40**).

From the stem bark of *T. wilfordii* var. *regelii* was isolated by Takaishi *et al.* [34] a new compound of this type, as an amorphous powder. This product showed a molecular formula $C_{29}H_{38}O_5$, and UV absorption bands typical of aromatic systems. The $^{1}$H-RMN spectrum of compound (**79**) was similar to that of 3-methyl-22β,23-dihydroxy-6-oxotingenol, previously isolated from *M. ilicifolia* and *M. chuchuasca* [84], and the

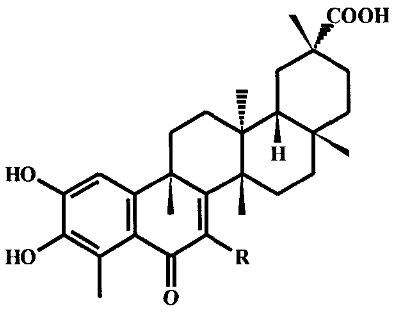
comparison of their $^{1}H$-NMR and $^{13}C$-NMR spectra allowed to establish the structure as 3-methyl-23-hydroxy-6-oxotingenol (**80**), Fig. (**41**).




**75**




**76**




**77** R = OH

**78** R = H

**Fig. (40).** Structures of zeylasterone ( **75**), regeol A ( **76**), regeol C ( **77**) and 23- *nor*-6-oxo-demethylpristimerol ( **78**).

From the root bark of *T. wilfordii* was isolated by Li *et al.* [26] a compound which exhibits a structure related to that of celastrol (**52**). It was named wilforic acid A (**79**), and its structure was established by applying spectroscopic methods. Besides IR absorption bands for hydroxyl (3402 $cm^{-1}$) and carbonyl (1702 $cm^{-1}$) groups the compound showed UV absorption bands at 249 and 286 nm. The $^{1}H$-NMR spectrum showed six methyl groups ($\delta$ 1.01 – 2.39 ppm), all of them as singlets,

and a proton attached to an aromatic ring (δ 7.07 ppm). $^{13}$C-NMR spectra were similar to that of two compounds discussed above in this review, regeol A (**76**), for carbon atoms C-1 to C-14 and C-25, and regeol B (**3**) for carbon atoms C-11 to C-23 and C26 to C-30. These facts, and the molecular formula $C_{29}H_{32}O_4$ obtained by HR-MS conducted to establish the structure of wilforic acid A (**79**) as a 24-*nor*-D:A-friedoleane triterpene bearing an cathecol moiety in ring A, Fig. (**41**).

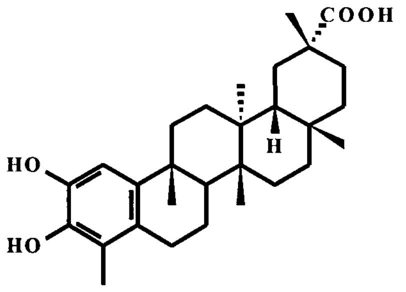


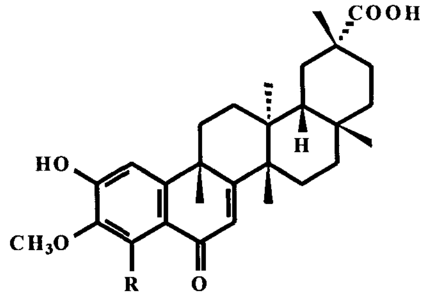

**Fig. (41).** Structure of wilforic acid A ( **79**).

The study of the outer root bark of *T. hypoglaucum* collected in China afforded three new structures of phenolic triterpenes. Duan *et al.* isolated triptohypol A (**80**), B (**81**) and C (**82**), a series of new phenolic compounds related to celastrol [29]. Triptohypol A (**80**) was isolated as an amorphous powder and spectroscopic means and chemical modification determined its structure. By HREIMS the formula $C_{30}H_{40}O_6$ was determined, and $^1$H-NMR spectrum showed five methyl signals, a methoxy moiety, a methylene attached to an oxygen atom and two vinyl protons. Data from $^{13}$C-NMR experiments indicated the presence of a pair of carbonyl groups and was quite similar to that of wilforol A (**78**). The analysis of HMQC and HMBC experiments allowed to establish the structure for rings A and B, based on the correlations observed between the proton H-1 (δ 6.95 ppm) and the carbons C-3, C-5 and C-10. Finally, the position of the carbonyl group was established at C-6 based on a NOESY experiment of the dimethyl derivative.

Triptohypol B (**81**) showed a very similar $^{13}$C-NMR spectrum when compared to triptohypol A, except for the replacement of a methylene

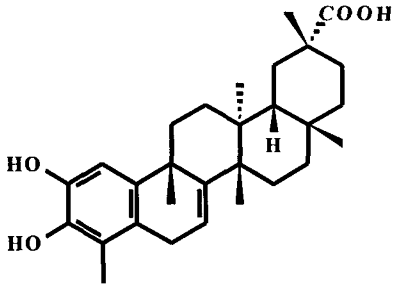
carbon by a methyl carbon, which was supported by the formula $C_{30}H_{40}O_5$, determined by HREIMS. A NOESY experiment indicated the correlation between the methyl proton signal with that of the C-3 methoxy group, which allowed to establish the structure of triptohypol B (**81**).

The structure of triptohypol C (**82**), was also determine by Duan *et al.* [29]. It was established after recognizing the presence of an aromatic system by UV absorption, and both carbonyl and hydroxyl groups by IR. The formula $C_{29}H_{40}O_4$ was determined by HREIMS and $^{13}C$-NMR data, which were similar to those of the wilforic acid A (**79**) isolated by Li *et al.* [26]. Great differences were observed in carbon signals of C-7, C-8 and C-25 when compared the spectra from triptohypol C and wilforic acid A, indicating differences in ring B. These observations and the identity of triptohypol C with the resulting product of the reduction of celastrol with sodium borohydride grant the structure elucidation of compound (**82**) as 2,3-dihydroxy-D:A-friedoleanan-1,3,5(10)-7-tetraene-29-oic acid, Fig. (**42**).




**80** R = $CH_2OH$

**81** R = $CH_3$




**82**

**Fig. (42).** Structures of triptohypol A (**80**), B (**81**) and C (**82**).

The chemical study *M. amazonica* roots [81] conducted to the identification of seven new phenolic triterpenes, besides the two new celastroloids discussed above. The structure of compound (**83**), isolated as yellow crystals, was determined by spectroscopy. HREIMS indicated a molecular formula $C_{28}H_{34}O_6$, and it showed IR absorption bands for carbonyl (1685, 1720 $cm^{-1}$) and hydroxyl (3550 $cm^{-1}$). $^{13}C$-NMR experiments indicated a phenolic triterpene related to 6-oxo-tingenol [85].

The position of both, the aldehyde group on C-23 and the hydroxyl on C-22 were established by HMBC experiments. The stereochemistry of the OH group was established as β based on the NOE effect observed between H-22 and the methyl 27 in a ROESY experiment. Compound (**83**) was identified as 2,3,22β-trihydroxy-24-29-dinor-1,3,5(10),7-friedelatetraene-6,21-dione-23-al, Fig. (**43**).

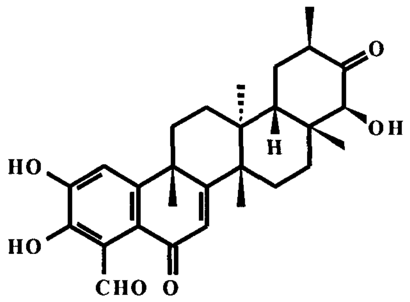



**Fig. (43).** Structure of compound ( **83**).

Compound (**84**), isolated as a yellow lacquer, showed a molecular formula $C_{28}H_{34}O_5$. Its $^1H$-NMR spectrum display similar characteristics as that of compound (**83**), except for the lack of the signal assigned to H-7 (δ 6.39 ppm) and the presence of three vinyl protons at δ 5.67, 6.70 and 6.74 ppm, all of them as doublets, which were assigned to H-11, H-6 and H-7. The analysis of data from $^{13}C$-NMR, HMBC and HMQC experiments confirmed the occurrence of one double bond in ring B (C-6, C-7) and other in ring C (C-9-C-11), establishing the structure of (**84**) as 2, 3, 22β-

trihydroxy-24-29-dinor-25 (9→8)-1, 3, 5(10), 7- friedelatetraene-21-one-23-al.

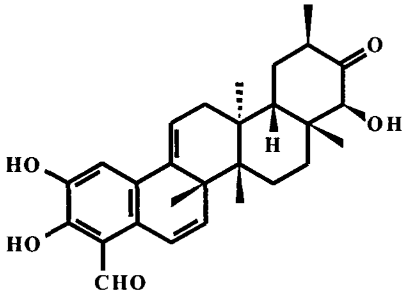
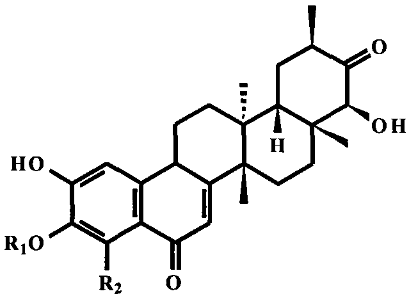



**Fig. (44).** Structure of compound ( **84**).

Compound (**85**) constitutes one of the minor components of the extract studied by Chávez *et al.* [81]. It was as an orange lacquer, showing a molecular formula $C_{28}H_{36}O_5$. Its $^1$H-NMR spectrum was similar to that of compound (**83**), except the lack of aldehyde signal, the presence of six methyl signals, one of them attached to an aromatic ring. With the aid of $^{13}$C-NMR data, the structure of (**85**) was established as 22β-hydroxy-3-methoxy-6-oxotingenol or 2, 22β-dihydroxy-3-methoxy-24-29-dinor-1,3,5(10),7- friedelatetraene-6,21-dione, Fig. (**45**).

The compound (**86**), isolated as yellow lacquer, exhibited a molecular formula $C_{27}H_{34}O_5$. Its structure was established mainly based on $^{13}$C-NMR, DEPT, and HMBC experiments, which showed a pair of carbonyl carbons (δ 213.53 s and 185.43 s), five methyl groups (δ 14.83 – 37.80), d two vinylic carbons conjugated to a carbonyl group and six aromatic carbons, four of them quaternary. $^1$H-NMR spectrum showed three singlets at low field (δ 7.79, 7.01 and 6.38 ppm) as observed in the spectrum of 23-*nor*-6-oxopristimerol, described by Gamlath and Gunatilaka [86] and assigned to H-4, H-1 and H-7, respectively. Based on these comparison the structure of (**86**) was established as 23-*nor*-22β-hydroxy-6-oxotingenol or 2, 3, 22β-trihydroxy-23,24,29-trinor-1,3,5(10),7- friedelatetraene-6,21-dione, Fig. (**45**).



85 $R_1 = H$, $R_2 = CH_3$

86 $R_1 = H$, $R_2 = H$

87 $R_1 = CH_3$, $R_2 = CH_3$

**Fig. (45).** Structure of compounds ( **85**), (**86**) and (**87**).

Compound (**87**) was also identified as a 6-oxotingenol derivative, and the spectral data were similar to those of (**85**), but showing a molecular formula $C_{29}H_{38}O_5$ by HREIMS. This was consistent with the presence of a signal in $^1$H-NMR assigned to a methoxy group (δ 3.81 ppm), which showed NOE effect with the methyl 23 in a ROESY experiment. From these data the structure of compound (**87**) was established as 22β-hydroxy-3-methoxy-6-oxotingenol, Fig. (**45**).

The compound (**88**) showed a $^1$H-NMR also similar to that of (**85**), but without the signals of the vinylic system. Besides that, the signals assigned to H-1 and the methyl 23 exhibited high field shifts as a result of the absence of the double bond in ring B, and consequently the change in the conformation of this ring. The structure of (**88**) was established as 22β-hydroxy-7,8-dihydro-6-oxotingenol, Fig. (**46**).

The last compound of this type isolated from *M. amazonica*, was also related to dihydrotingenol, showing a molecular formula $C_{29}H_{42}O_5$ (**89**). Its $^1$H-NMR spectrum showed one aromatic proton as a singlet (δ 6.65 ppm), a methoxy moiety (δ 3.42 ppm), six methyl signals (δ 0.86 – 2.57 ppm) being one a doublet (δ 1.08 $J$= 6.6 Hz), and a doublet at δ 4.62 ppm which is characteristic of the 22β-hydroxyl moiety in the tingenone skeleton. An extra hydroxyl group was located in C-15 according to the

chemical shift observed in 15α,22β-dihydroxy-tingenone described by Dias *et al.* [87]. Nevertheless, was impossible to obtain $^{13}$C-NMR data because of the unstable condition of the compound, it was proposed the structure 15α,22β-dihydroxy-7,8-dehydrotingenol for the compound (**89**), Fig. (**47**).

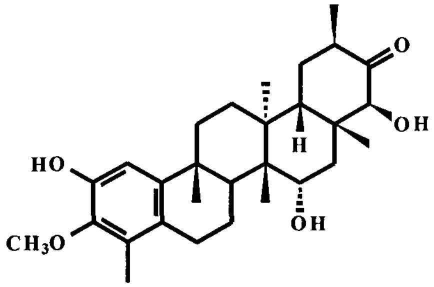



**Fig. (47).** Structure of compound ( **89**).

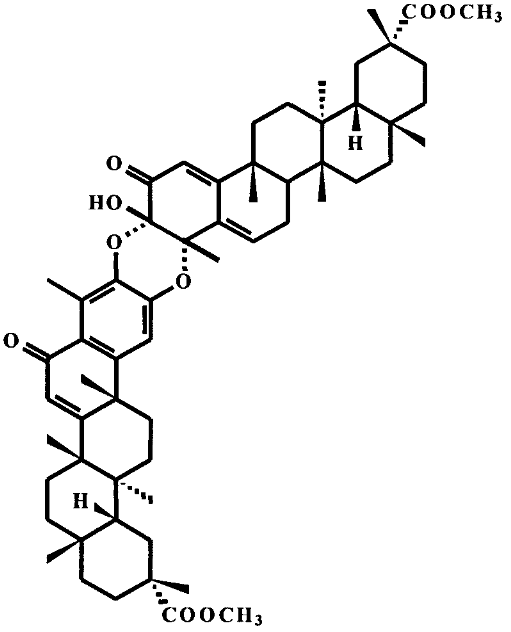
The authors also explain the biosynthesis of the new quinones and phenolic compounds described in the referred paper, by simple reactions using as the key compound 22β-hydroxytingenone, the most abundant secondary metabolite present in that extract.

## Triterpene dimers and trimers

Dimers are a class of compounds isolated from only seven species of Celastraceae family [8,88]. These triterpene dimers were found to be composed of one quinoid type and one aromatic type of triterpene derived from pristimerin, tingenone and / or their congeners joined together by two ether linkages formed between the two A rings or between the A and B rings. From the methylene chloride soluble portion of a methanolic extract of *Maytenus chuchuhuasca*, four dimeric compounds were isolated by Shirota *et al.* [88].

Compound (**90**) showed a molecular formula of $C_{60}H_{80}O_9$, determined by HRFAB-MS. The $^{1}$H and $^{13}$C NMR spectra of (**90**) showed that two pristimerin-type triterpene units composed it, one in the quinoid form and the other in the aromatic form. The units were different that those from scutidin αA [89] and xuxuarine Eβ [90]. From the spectroscopic data, it

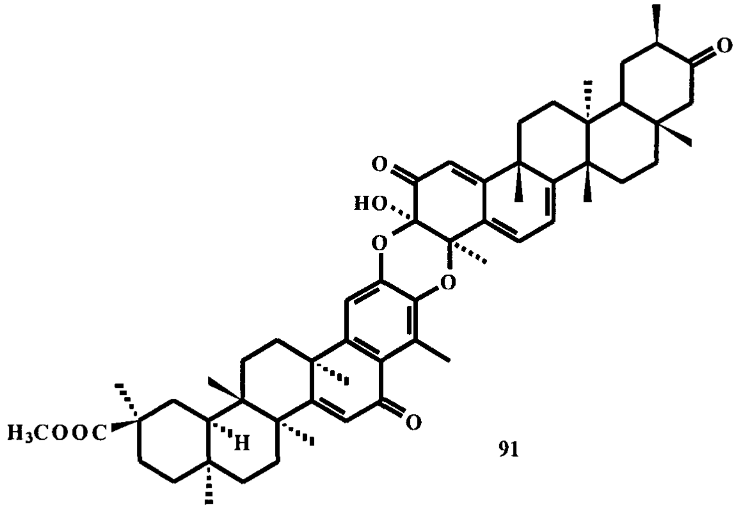
was suggested that the conjugated ketone system on the A and B ring of the quinoid triterpene unit was partially saturated between C-7 and C-8. The structure was confirmed by 2D NMR spectroscopy and the structure of (**90**) is showed in Fig. (**48**).



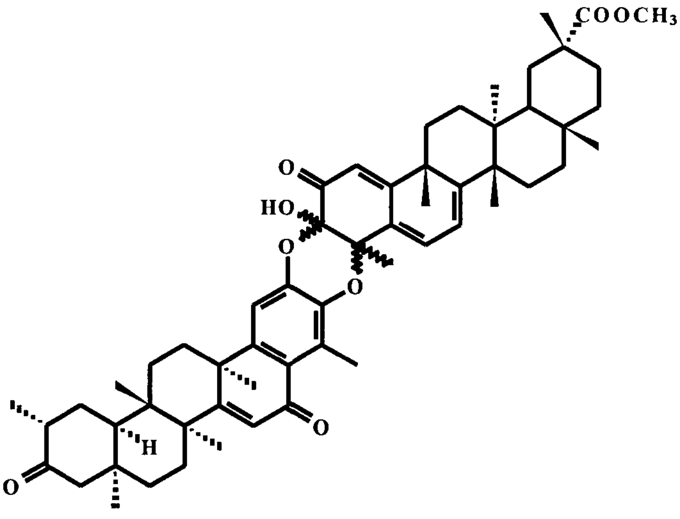

Shirota, Morita, Takeya and Itokawa (1998)

**Fig. (48).** Structure of compound ( 90)

Compounds (**91**), (**92**) and (**93**) had an identical molecular formula $C_{58}H_{74}O_8$, which was established by HRFAB-MS. The NMR spectral data, which were similar to those of cangorosin B [7], suggested that each consisted of two different types of triterpene units, tingenone and pristimerin, one in the quinoid form and the other in the aromatic form. The stereochemistry was confirmed by means of ROESY and CD spectral studies. The FAB-MS data enabled to determine whether the pristimerin and the tingenone type triterpene were in the quinoid form or in the aromatic form. Considering the spectroscopic evidences, the structures of (**91**), (**92**) and (**93**) were assigned to xuxuarines Fβ, Gα and Gβ respectively, Fig. (**49**).




91



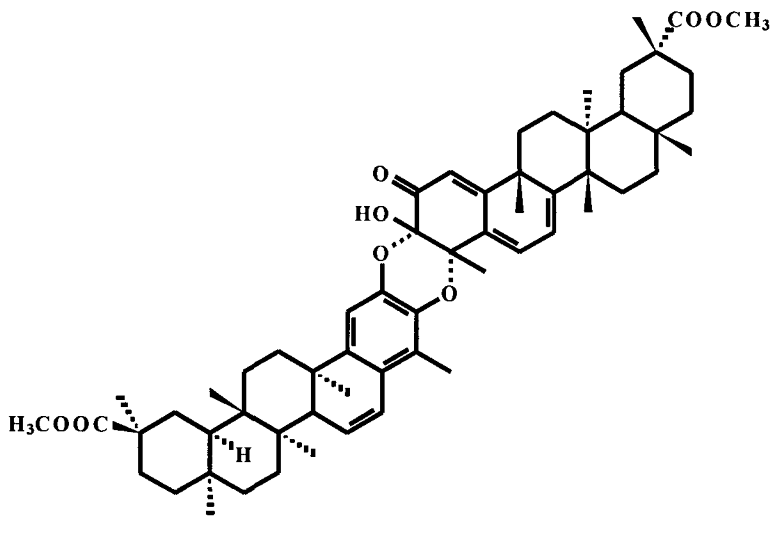

92 = *cis* 3,4-dioxy bond = α

93 = *cis* 3,4-dioxy bond = β

Fig. (49). Structures of compounds (91), (92) and (93)

From *Maytenus blepharodes* and *M. magellanica*, three dimeric compounds based on two pristimerin units were isolated by González *et al.* [91].

Compound (**94**) showed a molecular formula of $C_{60}H_{80}O_8$, based on HRFAB-MS analysis and its $^{13}$C-NMR spectrum. According to the data obtained from its $^1$H and $^{13}$C-NMR spectra suggested that (**94**) was a triterpene dimer composed of two pristimerin-type triterpenes with one subunit in the quinoid form and the other in the aromatic form. Confirmation of the structure was made by analysis of the 2D NMR spectroscopic data. The absolute configuration was determined from its CD spectrum. According to the above data, the authors concluded that (**94**) was a regioisomer of scutionin αA [89], and it was called scutionin αB, Fig. (**50**).




González, Kennedy, Rodríguez, Bazzocchi, Jiménez, Ravelo and Moujir (2001)

**Fig. (50).** Structure of scutionin αB (94)

Compound (**95**) showed a molecular formula of $C_{60}H_{82}O_8$, also by HRFAB-MS and its $^{13}$C-NMR spectrum. The main difference with (**94**) was the absence of the C-6'-C-7' double bond, confirmed by 2D NMR spectroscopy. The absolute configuration was determined-as in the former case-by analysis of its CD spectrum. According to the data obtained, the structure of (**95**) was assigned as 6',7'-dihydro-scutionin αB, Fig. (**51**).

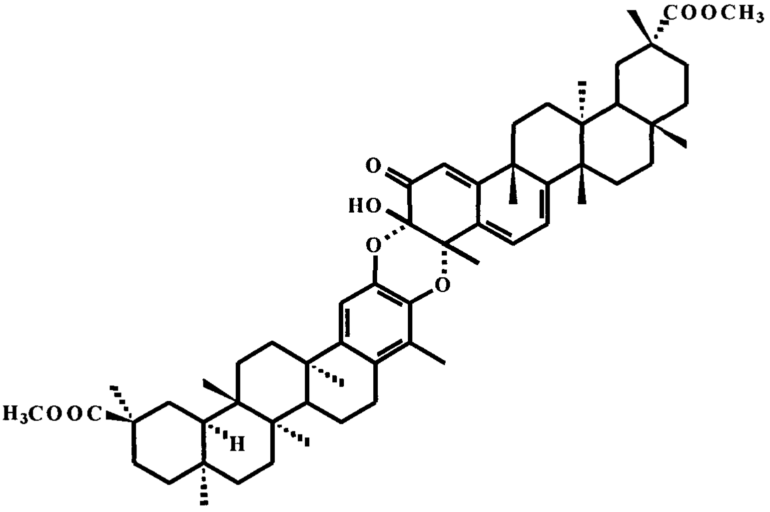



Fig. (51). Structure of 6',7'-dihydro-scutionin αB (95)

Compound (**96**) showed to be a dimeric triterpene based on its spectral data and showed a molecular formula of $C_{61}H_{84}O_9$, based again on its

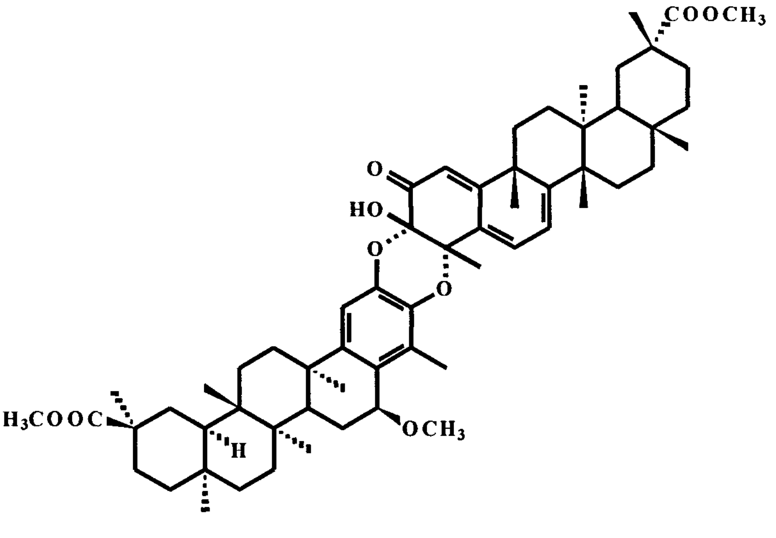
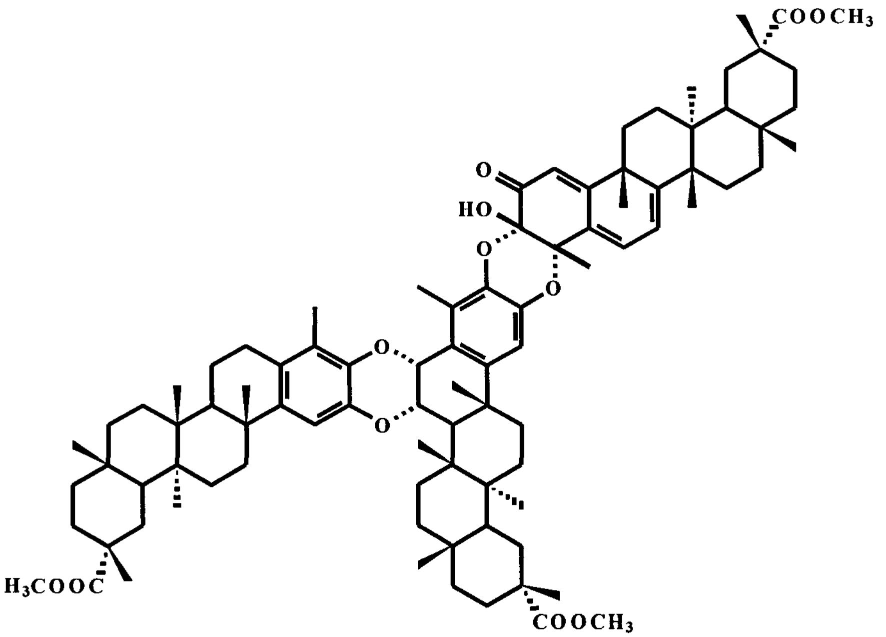



Fig. (52). Structure of 6' β-methoxy-dihydro-scutionin αB (96)




González, Alvarenga, Bazzocchi, Ravelo and Moujir (1999)

**Fig. (53).** Structure of triscutin A ( 97)

HRFAB-MS and $^{13}$C-NMR spectrum. The most notable differences with compound (**94**), was the absence of the C-6'-C-7' double bond and an additional methoxy group, that was located at C-6', according to a HMBC experiment. As in the case of the preceding compounds, the structure was confirmed by 2D NMR analysis and CD determined the absolute configuration. With those data, the authors determined the structure of (**96**) as 6'β-methoxy-dihydro-scutionin αB, Fig. (**52**).

Trimeric compounds have only been isolated from one species of the Celastraceae family, *M. scutioides*, by González *et al.* [8]. The two compounds were based on pristimerin units, one in the quinoid form and two in the aromatic form.

Compound (97) showed a molecular formula of $C_{90}H_{122}O_{12}$, based on its FAB-MS and $^{13}$C-NMR spectra. From its $^{1}$H and $^{13}$C-NMR spectra, the compound showed four ether linkages joining the three terpene units. According to its 2D NMR spectroscopic data, the two diether linkages were determined to be C3-O-C3, C4-O-C2', and C6'-O-C3'', C7'-O-

C2'', respectively. The absolute stereochemistry was determined by analysis of its CD spectrum. In accordance with these data, the structure of (**97**) is showed above, and the compound was called triscutin A, Fig. **(53)**.

Compound (**98**) showed a molecular formula of $C_{90}H_{122}O_{12}$, according with its FAB-MS and [13]C-NMR spectra. Analysis of the data obtained from the [1]H-NMR spectrum and the 2D NMR experiments, showed that (**98**) was an isomer of (**97**), with different substitution of the subunits. Its absolute configuration was determined by its CD curve. The lingkages between the units were determined to be C3-O-C2', C4-O-C3' and C6'-O-C2'', C7'-O-C3'', respectively. According to the data obtained, the structure of (**98**) is showed below, and the compound was called triscutin B, Fig. **(54)**.

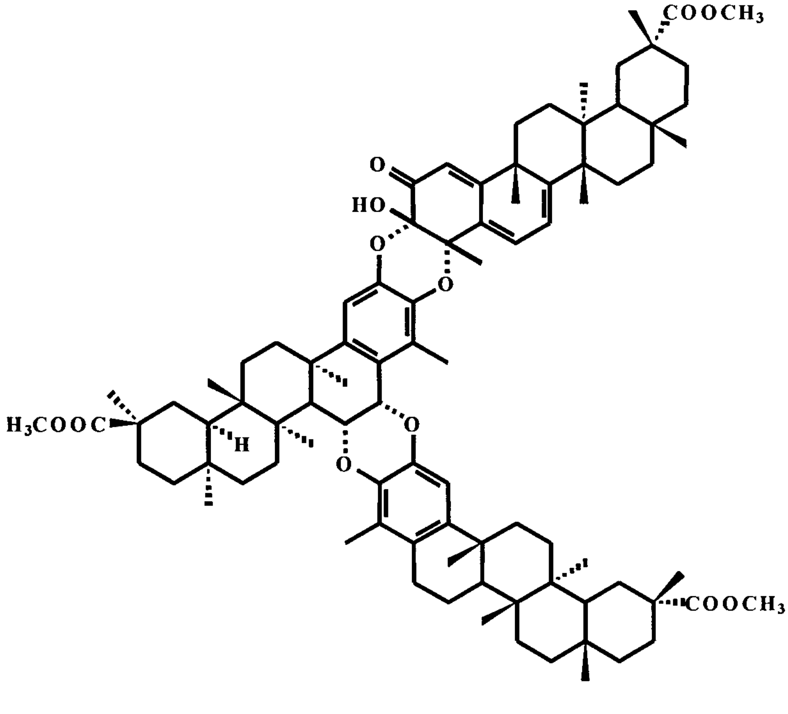



**Fig. (54).** Structure of triscutin B ( 98)

## BIOLOGICAL ACTIVITIES

A wide range of biological activities has been described for triterpenes and nor-triterpene quinonemethides. These include anti-inflammatory, anti-hyperlipidemic, antitumour-promotion, hepatoprotective, anti-ulcer, anti-microbial and hypoglycemic activitiy, among others [16,35]. Among the compound mentioned previously in this review, antitumour-promotion, anti-inflammatory, inmunosuppressive, anti-tumour, insecticide and anti-AIDS activities were found and are described below.

### Anti-inflamatory Activity

*T. wilfordii* has been used as a traditional medicine in China for many centuries. The plant has also been used to treat rheumatoid arthritis and spondylitis [92]. It has been reported that there is a strong relationship between the production of interleukin – 1 (IL-1) by the synovium and the degree of inflammation of the arthricular synovial membrane in rheumatoid artritis [93-95]. Regeol A (**76**), B (**3**) and C (**77**) [27], isolated from *T. wilfordii* var. *regelii* showed moderate to low inhibitory activity for IL-1$\alpha$ and IL-1$\beta$ release from lipopolysaccharide-stimulated human peripheral mononuclear cells compared to prednisolone as a reference compound.

From an extract derived from the water/chloroform extract of the roots of *T. wilfordii* triterpenes and phenolic triterpenes with potential anti-inflammatory activity were also isolated [33]. The compounds were assayed for inhibitory effects on cytokine production. Despite the fact that the compounds that showed the highest inhibitory levels were diterpenes, the triterpenes showed interesting percentages of inhibitory activity. The degree of oxidation in the A-ring seems to play an important role in the inhibitory activity level, mainly in the production of IL-2, IL-4 and interferon gamma (IFN-$\gamma$). Supporting this assumption are the fact that compounds (**20**) and (**99**)-that has two hydroxyl groups in the A-ring-showed the highest inhibitory levels on the production of IL-2, IL-4 and IFN-$\gamma$ among the triterpenes, isolated from the extract. Compound (**99**) – which was not previously, discussed-showed a structure very similar of that of wilforic acid A (**79**), except for the presence of a hydroxyethyl moiety at C-6, Fig. (**55**). The inhibitory effects of the compounds are summarized in Table 1. Compound (**99**) showed inhibitory activity higher

than prednisolone. This compound, in conjunction with the diterpenes was pointed out to be responsible for the immunosuppressive activity of the extract.

**Table 1. Inhibitory effects of compounds isolated from *T. wilfordii* and *T. wilfordii* var. *regelii* [27,33]**

| Compound | Inhibition (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | IL-1α | IL-1β | IL-8 | TNF-α | IL-2 | IL-4 | IFN-γ |
| **3** | 1 | 0 | - | - | - | - | - |
| **20** | - | -10 | 40 | -1 | 100 | 56 | 68 |
| **22** | - | -4 | 46 | 9 | 53 | 34 | 1 |
| **24** | - | -25 | -28 | 24 | 63 | 38 | 10 |
| **30** | - | -69 | -84 | -7 | 70 | 36 | 20 |
| **99** | - | 92 | 83 | 100 | 100 | 100 | 100 |
| **Prednisolone** | - | 68 | 15 | 52 | 65 | 76 | 75 |

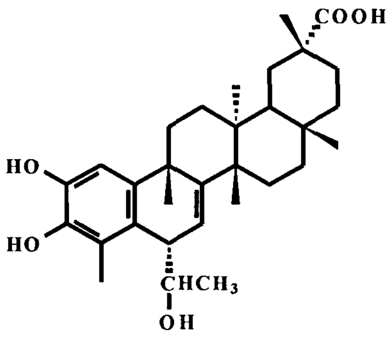



Fig. (55). Structure of compound ( 99)

## Antitumor activity

Compounds isolated from Celastraceae had showed cytotoxic activity [3,4]. This property has been related mainly to nor-triterpene quinonemethides, but cytotoxic triterpenes had also been reported [96]. As an example, both, tumor initiation and promotion are inhibited by two triterpenes commonly isolated from the familiy, ursolic and oleanolic acids [16].

Four compounds isolated from *C. hindsii* [37], maytenfolone-A (**10**), celasdin A (**11**), celasdin B (**12**) and celasdin C (**13**) were tested for cytotoxicity in four cancer cell lines. The lines used were KB (nasopharynx carcinoma), HEPA-2B (hepatoma), HELA (cervix carcinoma) and COLO-205 (colon carcinoma). Compound (**10**) showed

cytotoxicity against hepatoma (HEPA-2B, $ED_{50}$ = 2.3 μg $mL^{-1}$) and nasopharynx carcinoma (KB, $ED_{50}$ = 3.8 μg $mL^{-1}$) cells, whereas the other compounds were inactive, compared with the reference compound, mitomycin C against the above four cell lines. The authors suggested that-according these results-the friedelin derived seven-membered ring lactone between C-17 and C-20 may be important if the hydroxyl is located at C-16.

*M. amazonica*, is distributed in the amazonian region of Peru and are reputed as a therapeutic agent in the treatment of rheumatism, flu, gastrointestinal diseases and as an antitumoral agent for skin cancer [97-98]. The phenolic triterpenes isolated from this source [81] and previously described in this review were assayed for cytotoxic activity against P-388 (mouse lymphoma), A-549 (human lung carcinoma), HT-29 (human colon carcinoma) and MEL-28 (human melanoma). The results showed in Table 2, according to authors, seems to indicate that the aldehyde group at C-23 and the 6-oxo-α,β-unsaturated systems in the B ring are important for a high activity.

**Table 2. Cytotoxic activity from compounds isolated from *M. amazonica* [81]**

| | $IC_{50}$ μg $mL^{-1}$ | | | | |
|---|---|---|---|---|---|
| | **83** | **84** | **86** | **87** | **88** |
| **P-388** | 0.25 | 2.5 | 10 | 1.0 | 2.5 |
| **A-549** | 0.25 | 5.0 | 10 | 1.0 | 2.5 |
| **HT-29** | 0.50 | 5.0 | 10 | 1.0 | 2.5 |
| **MEL-28** | 0.50 | 5.0 | 10 | 1.0 | 2.5 |

*C. gaumeri* is used for treatment of snake bite in the form of a plaster and the decoction is used orally for diarrhea. From this source, Ankli *et al.* isolated cardenolides-previously discussed-with cytotoxic activity against the KB cell line [59]. The summarized data are showed in Table 3. According to the authors, it is worthy of note that the cytotocicity is not correlated with the oxidation status of the C-19 methyl group. Comparison between compounds with identical genin and different sugar chain revealed a significant difference. Introduction of a hydroxyl group at C-5 doubled the cytotoxicity.

**Table 3. Cytotoxic activity of compounds isolated from *C. gaumeri* [59] against KB cell line**

| Compound | $IC_{50}$ | Standard error | *n* |
|---|---|---|---|
| **47** | 0.25 | 2.5 | 10 |
| **48** | 0.25 | 5.0 | 10 |
| **49** | 0.50 | 5.0 | 10 |
| **50** | 0.50 | 5.0 | 10 |

15α-hydroxy-pristimerin (**57**), isolated from *M. scutioides* was previously discussed [72]. This compound was assayed for cytotocicity against HeLa (human carcinoma of the cervix) and Hep-2 (human carcinoma of larynx). The results obtained-using pristimerin (**53**) as comparison substance-showed that compound (**57**) was slightly less active as cytotoxic than pristimerin. Having in mind the structures of both compounds, it is clear that the hydroxyl group, located at C-15 has an unfavorable effect on the activity of the compound. The data is showed in Table 4.

**Table 4. Cytotoxic activity of 15α-hydroxy-pristimerin compared with pristimerin [71]**

| | $IC_{50}$ μg $mL^{-1}$ | |
|---|---|---|
| | **53** | **57** |
| **HeLa** | 0.30 | 1.2 |
| **Hep-2** | 1.04 | 3.1 |

Triterpene dimers and trimers discussed in the present review probed to be inactive as cytotoxic [91,8]. According to González *et al.*, this fact is due that the size of this type of compound plays an important role in their activity and that they could be stored in the plant as polymers, which could release biologicaly active units, like the quinone methides.

## Antimicrobial activity

Among the compounds previously described, some of them showed antimicrobial activity. All the active compounds belong to the quinone methide-type. A phenolic compound isolated by Ankli and coworkers and not previously discussed (**100**), Fig. (**56**), was also tested, but it showed very low activity.

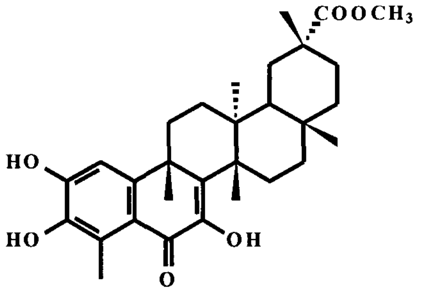



Fig. (55). Structure of compound ( **100**)

Compounds (**57**) and (**58**), isolated from *Maytenus* species [72,73], were tested against several Gram-positive and Gram-negative bacteria and the yeast *Candida albicans*. Both compounds were more active than pristimerin (**53**) and, in the case of (**57**), than cephotaxime against certain strains. Those data support the hypothesis of González [99] that the oxidation grade in the molecule increased the antimicrobial activity in the nor-triterpene quinone methides. The compounds were inactive against the Gram-negative bacteria and the yeast. The data are showed in Table 5.

**Table 5. Minimal inhibitory concentrations (MIC, μg mL$^{-1}$ of (57) and (58) against Gram (+) bacteria [72,73]**

| Compound | Bacteria | | | | | | |
|---|---|---|---|---|---|---|---|
| | *S. aureus* | *S. epidermidis* | *S. saprophyticus* | *S. warnieri* | *M. luteus* | *B. subtilis* | *B . pumilus* |
| **53** | >50 | 2.5 | >50 | >50 | 18-16 | 1-0.5 | 2-1 |
| **57** | 6-5 | 1.25-0.6 | 10-5 | 5-2.5 | 2-5 | 2-1 | 3-2 |
| **58** | 5.0 | - | - | - | - | 1.25-2.5 | - |
| **cephotaxime** | 2.5-1.25 | 5-2 | 0.62-0.31 | 1.25-0.62 | 0.06-0.03 | 8 | >10 |

Cephotaxime was used as control only with compound (57).

*S. krausii* is used in Mozambican traditional medicine against bilharziasis and dysentery. However, Celastraceae plants were also reported to possess antimalarial activity [35]. Figueiredo *et al.* isolated three nor-triterpene quinone methides [77], compounds (**60**), (**61**) and (**62**). The *in vitro* activity (expressed as $IC_{50}$ values) of the isolated quinone methides in comparison to the reference compound (chloroquine) is shown in Table 6. The isolated compounds, together with the known celastroloids celastrol (**52**), pristimerin (**53**) and isoiguesterol (**101**), Fig.

(**56**) were assayed against a multidrug-resistant strain *K1* and a drug-sensitive strain $NF_{54}$ of *Plasmodium falciparum*. The authors found that the activity of all compound except 28-*nor*-isoiguesterin-17-carbaldehyde (**61**) was slightly higher against the multidrug –resistant strain *K1* than against the drug-sensitive strain $NF_{54}$ of *P. falciparum*. Compounds with the best activities were isoiguesterol (**101**) and 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**62**). The other compounds showed lower levels of activity and all the compounds assayed showed lower *in vitro* activity against the drug-sensitive strain $NF_{54}$ than the reference compound, chloroquine (10-50 times lower). Nevertheless, 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**62**) and isoiguesterol (**101**) were two to three times more active than chloroquine against the multidrug-resistant strain *K1*. Compound showed to be some more active against the *Plasmodium* strains than against the human adenocarcinoma cell line HT-29 (10-100 fold more active). Parenteral administration of 17-(methoxycarbonyl)-28-*nor*-isoiguesterin lead to death of the animals assayed (10 mg/kg body weight) after 1 day, confirmed previous studies that describes triterpenoids quinone methides as toxic products [35]. Lower parenteral doses (1 and 5 mg/kg ) and 30 mg/kg peroral produce no effects over the parasitaemia. *In vitro* drug interaction studies were also undertaken, in order to detect synergistic, additive or antagonistic effects within the compounds. Celastrol (**52**) and pristimerin (**53**) showed an additive effect. The compounds (**62**) and (**101**) showed slightly antagonistic effect against strain *K1*. When assayed with chloroquine, compound (**62**) showed a highly antagonistic effect against $NF_{54}$ strain and a slightly antagonistic effect against the multidrug-resistant strain *K1*.

**Table 6. Antiplasmodial activity of the quinone methides isolated from the roots of *Salacia kraussii* [77]**

| | $IC_{50}$ (ng $ml^{-1}$) | |
|---|---|---|
| **Compound** | *P. falciparum K1* | *P. falciparum $NF_{54}$* |
| **52** | 180.9 | 254.2 |
| **53** | 190.4 | 270.9 |
| **60** | 114.4 | 140.2 |
| **61** | 94.0 | 79.9 |
| **62** | 27.6 | 37.1 |
| **101** | 22.9 | 54.1 |
| **Chloroquine** | 61.1 | 4.6 |

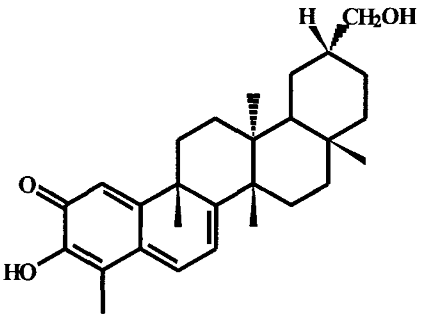



**Fig. (56)**Structure of isoiguesterol (**101**)

## Inhibition of tumor production

In recent years, the anti-tumor-promoting activities of several natural products [100] have been investigated for inhibition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced Epstein-Barr virus early antigen (EBV-EA) activation [101]. Compounds isolated from *T. wilfordii* var. *regelii* were assayed by Takaishi and coworkers [27] on EBV-EA activation. Their inhibitory effects on the activation of the early antigen and the viabilities of Raji cells (in parenthesis) are shown in Table 7. Regeol B (**3**) showed higher activity than regeol C (**77**).

**Table 7. Inhibition of the EBV-EA activation by constituents of *T. wilfordii* and their derivatives [27]**

| | Concentration * | | | |
|---|---|---|---|---|
| **Compound** | 1 x $10^3$ | 5 x $10^2$ | 1 x $10^2$ | 1 x10 |
| 3 | 0 (70) | 53.7 (>80) | 83.6 | 100 |
| 77 | 11.3 (70) | 65.4 (>80) | 84.5 | 100 |

* Mol ratio / TPA (20 ng $mL^{-1}$ = 32 pM $mL^{-1}$

Activity is presented relative to a positive control (Induction (100%) by TPA at 20 ng $mL^{-1}$ )

## Insecticidal activity

Products isolated from plants of the Celastraceae family have showed insecticidal and antifeedant activities, as discussed earlier. The compounds reported belong to the agarofuran sesquiterpene-type and sesquiterpene pyridine-type alkaloids. Nevertheless, the only description of insecticidal activity of quinone methides found is from Avilla *et al* [102]. Despite the fact that the compounds assayed were known the

results were very interesting, showing that the studies of quinone methides as insecticidal and antifeedant compounds should be intensified. The compounds evaluated were pristimerin (**53**), tingenone (**54**) and 20α-hydroxy-tingenone (**102**), Fig. (**57**). The work determined the effect of the three compounds on the mortality, feeding activity, and development of larvae of codling moth *Cydia pomonella* (L.), (Lepidoptera: Tortricidae).

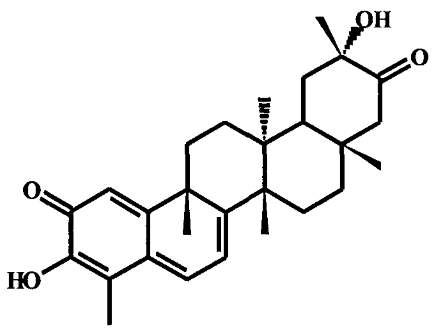



**Fig. (57).** Structure of 20α-hydroxy-tingenone (**102**)

The three metabolites assayed produced the same effect on the insect used that the control (a commercial extract of the neem tree, containing 2% of azadirachtin), although at higher concentrations. 20α-hydroxy-tingenone was the most active compound among the three assayed. Pristimerin shows also a high antifeedant activity together with molt effect suppression.

Tingenone was the compound with the lowest activity. The importance of those type of compounds is supported by the fact that insects resistant to some products actually in use could constitute a potential risk for the crops and, also, the use of not biodegradable chemicals for the control of pest are a menace to the environment.

**Table 8. Mortality of *C. pomonella* larvae after 5 or 10 days of continuous exposure to diet topically treted with 50 μL of a solution of compounds (53), (54) and (102) [102][a]**

| Concn (mg mL$^{-1}$) | 53 | | 54 | | 102 | |
|---|---|---|---|---|---|---|
| | 5 days | 10 days | 5 days | 10 days | 5 days | 10 days |
| 0 | 0.0 ± 0.0 c | 3.3 ± 3.3 c | 0.0 ± 0.0 a | 3.3 ± 3.3 a | 0.0 ± 0.0 | 3.3 ± 3.3 |
| 1 | | | | | 3.3 ± 3.3 | 3.3 ± 3.3 |
| 2 | | | | | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 4 | | | | | 6.7 ± 3.3 | 16.7 ± 6.7 |
| 6 | | | | | 10.0 ± 10.0 | 23.3 ± 18.6 |
| 8 | | | | | 3.3 ± 3.3 | 43.3 ± 12.0 |
| 12 | | | | | 53.3 ± 14.5 | 60.0 ± 11.5 |
| 16 | | | | | 60.0 ± 5.8 | 93.3 ± 3.3 |
| 18 | | | | | 86.7 ± 6.7 | 93.3 ± 6.7 |
| 20 | 20.0 ± 15.3 bc | 60.0 ± 15.3 b | 0.0 ± 0.0 a | 0.0 ± 0.0 a | 100.0 ± 0.0 | 100.0 ± 0.0 |
| 40 | 33.3 ± 3.3 b | 60.0 ± 10.0 b | 3.3 ± 3.3 a | 10.0 ± 5.8 a | | |
| 80 | 90.0 ± 5.8 a | 100.0 ± 0.0 a | 13.3 ± 8.8 a | 23.3 ± 12.0 a | | |
| $LC_{50}$ | | | | | 13.0 | 8.5 |

[a] Mean and SE of the percentage of dead larvae, calculated from 3 replicates of an initial number of 10 larvae each. Means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD test). $LC_{50}$ calculated by means of probit analysis.

## Aldose reductase inhibition

The roots of *S. oblonga* have been extensively used for the treatment of diabetes in the Ayurvedic system of traditional Indian medicine. From the ethyl acetate soluble portion of the methanolic extract from the roots of *S. oblonga* Matsuda *et al.* isolated compound (**14**), a friedelane-type triterpene [42]. The methanolic extract and the ethyl acetate soluble portions were found to show inhibitory activity on aldose reductase, which is related to chronic diabetes complications such as peripheral neuropathy, retinopathy and cataracts. Compound (**14**) was assayed for aldose reductase rat lens inhibitory activity. It showed inhibition percentages of 21.8 % at a concentration of 30 μM and 48.2 % at a concentration of 100 μM. The activity of (**14**) and other terpenoids isolated from this plant could prove to be the reason for the popular use of the plant in the Ayurvedic system for the treatment of diabetes.

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## REFERENCES

[1] Loub, W.D.; Farnsworth, N.R.; Soejarto, D.D.; Quin, M.L.; *J. Chem. Inf. Comput. Sci.*, **1985**, *25*, 99.

[2] Brüning, R.; Wagner, H.; *Phytochemistry*, **1978**, *17*, 1821-1858.

[3] González, A.G.; Jiménez, I.A.; Ravelo, A.G.; Bazzocchi, I.L.; *Tetrahedron*, **1993**, *49*, 697-702.

[4] González, A.G.; Ravelo, A.G.; Bazzocchi, I.L.; Jiménez, J.; Gomzález, C.M.; Luis, J.G.; Ferro, E.A.; Gutiérrez, A.; Moujir, L.; Las Heras, F.G.; *Il Fármaco*, **1988**, *43*, 451-455.

[5] Hegnauer, R.; *Chemotaxonomie der Pflanzen*, Basel: Birkhäuser, **1996**.

[6] Itokawa, H.; Shirota, O.; Morita, H.; Takeya, K.; Tomioka, N.; Itai, A.; *Tetrahedron Lett.*, **1990**, *31 (47)*, 6881-6882.

[7] Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H.; *J. Nat. Prod.*, **1997**, *60*, 111-115.

[8] González, A.G.; Alvarenga, N.L.; Bazzocchi, I.L.; Ravelo, A.G.; Moujir, L.; *J. Nat. Prod.*, **1999**, *62 (8)*, 1185-1187.

[9] Bhatnagar, S.S.; Divekar, P.V.; *J. Sci. Industr. Res.* **1951**, *10B*, 56.

[10] Schwenk, E.; *Arzneim-Forsch*, **1962**, *12*, 1143.

[11] Pavanand, K.; Webster, H.K.; Yongvanitchit, K.; Kun-Anake, A.; Dechatiwongse, T.; Nutakul, W.; Bansiddhi, J.; *Phytother. Res*, **1989**, *3*, 136.

[12] Heywood, V.H.; *Flowering Plants of the World*, Oxford University Press: New York, **1993**.

[13] Woodland, D.W.; *Contemporary Plant Systematics*, Prentice-Hall: New Jersey, **1991**.

[14] Robson, N.; *Bot. Soc. Brot.,Ser.*, **1965**, *2 (39)*, 5.

[15] Simmons, M.P.; Clevinger, C.C.; Savolainen, V.; Archer, R.H.; Mathews, S.; Doyle, J.J.; *Am. J. Bot.*, **2001**, *88*, 313, 325.

[16] Liu, J.; *J. Ethnopharmacol.*, **1995**, *49*, 57-68.

[17] Corsino, J.; Alécio, A.C., Ribeiro, M.L.; França, S.C.; Pereira, A.M.S.; Duarte, I.B.; Furlan, M.; *Phytochem. Anal.*, **1998**, *9*, 245-247.

[18] Gunaherath, G.M.K.B.; Gunatilaka, A.A.L.; *J. Chem. Soc. Perkin Trans I*, **1983**, *24*, 2845-2847.

[19] Gunatilaka, A.A.L.; Nanayakkara, N.P.D.; *Tetrahedron*, **1984**, *40*, 805-809.

[20] Kutney, J.P.; Beale, M.H.; Salisbury, P.J.; Stuart, K.L.; Worth, B.R.; Towsley, P.M.; Chalmers, W.T.; Nilsson, K.; Jacoli, G.C.; *Phytochemistry*, **1981**, *20*, 653-657.

[21] Marini-Bettolo, G.B.; *Rev. Latinoamer. Quim.*, **1979**, *10*, 97-104.

[22] Corsino, J.; de Carvalho, P.R.; Kato, M.J.; Ribeiro, L.; Oliveira, O.M.M.F.; Araujo, A.R.; da S. Bolzani, V.; França, S.; Pereira, A.M.; Furlan, M., *Phytochemistry*, **2000**, *55*, 741-748.

[23] Kupchan, S.M.; Cout, W.A.; Dailey, R.G.; Gilmore, C. Jr.; Bryan, R.F.; *J. Amer. Chem. Soc.*, **1972**, *94*, 7192.

[24] Kutney, J.P.; Hewitt, G.M.; Kurihara, T.; Salisbury, P.J.; Sindelar, R.D.; Strurt, K.L.; Townley, P.M.; Chalmers, W.T.; *Can. Jour. Chem.* **1981**, *59*, 2677.

[25] Chen, K.; Shi, Q.; Kashiwada, Y.; Zhang, D.-C.; Hu, C.-Q.; Jin, J.-Q.; Nozaki, H.; Kilkuskie, R.E.; Tramontao, E.; Cheng, Y.-C.; Mcphail, D.R.; Mcphail, T.; Lee, K.-H.; *J.Nat. Prod.*, **1993**, *55*, 340.

[26] Li, K.; Duan, H.; Kawazoe, K.; Takaishi, Y.; *Phytochemistry*, **1997**, *45 (4)*, 791-796.

[27] Takaishi, Y.; Wariishi, N.; Tateishi, H.; Kawazoe, K.; Nakano, K.; Ono, Y.; Tokuda, H.; Nishino, H.; Iwashima, A.; *Phytochemistry*, **1997**, *45 (5)*, 969-974.

[28] Itokawa, H.; Shirota, O.; Ikuta, H.; Morita, H.; Takeya, K.; Iitaka, Y.; *Phytochemistry*, **1991**, *30*, 3713.

[29] Duan, H.; Kawazoe, K.; Bando, M.; Kido, M.; Takaishi, Y.; *Phytochemistry*, **1997**, *46 (3)*, 535-543.

[30] Nakano, K.; Oose, Y.; Takaishi, Y.; *Phytochemistry*, **1997**, *46 (7)*, 1179-1182.

[31] Nakanishi, K.; Gullo, V.P.; Miura, I.; Govindachari, T.R.; Viswanathan, N.; *J. Amer. Chem. Soc.*, **1973**, *95 (19)*, 6473.

[32] Yang, G-Z.; Li, Y-c.; *Helv. Chim. Ac.*, **2002**, *85*, 168-174.

[33] Duan, H.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T.; Tori, M.; Takaoka, S.; Jia, Y.; Li, D.; *Tetrahedron*, **2001**, *57*, 8413-8424.

[34] Takaishi, Y.; Miyagi, K.; Kawazoe, K.; Nakano, K.; Li, K.; Duan, H.; *Phytochemistry*, **1997**, *45 (5)*, 975-978.

[35] Gunatilaka, A.A.L.; *Progress in the Chemistry of Organic Natural Compounds*, **1996**, *67*, 1.

[36] Gamlath, C.B.; Gunatilaka, A.A.L.; Tezuka, Y.; Kikuchi, T.; Balasubramaniam; *Phytochemistry*, **1990**, *29*, 3189.

[37] Kuo, Y-H.; Yang, L-M.; *Phytochemistry*, **1997**, *44 (7)*, 1275-1281.

[38] Gunasekera, S.P.; Sultanbawa, M.; *J.Chem. Soc., Perkin Trans. I.*,**1977**, 418.

[39] Nozaki, H.; Suzuki, H.; Hirayama, T.; Kasai, R.; Wu, R.Y.; Lee, K.H.; *Phytochemistry*, **1986**, *25*, 479.

[40] Budzikiewicz, H.; Wilson, J.M.; Djerassi, C.; *J. Amer. Chem. Soc.*, **1963**, *85*, 3688.

[41] Sengupta, P.; Chakraborty, A.K.; Duffield, A.M.; Durham, L.J.; Djerassi, C.; *Tetrahedron*, **1967**, *24*, 1205.

[42] Matsuda, H.; Murakami, T.; Yashiro, K.; Yamahara, J.; Yoshikawa, M.; *Chem. Pharm. Bull.*, **1999**, *47 (12)*, 1725-1729.

[43] Chen, B.; Duan, H.; Takaishi, Y.; *Phytochemistry*, **1999**, *51*, 683-687.

[44] Alvarenga, N.; Ferro, E.A.; *Fitoterapia*, **2000**, *71*, 719-721.

[45] Fujita, R.; Duan, H.; Takaishi, Y.; *Phytochemistry*, **2000**, *53*, 715-722.

[46] Zhang, C.-P.; Zhang, Y.-G.; Lu, X-Y.; Chen, Y.; Ma, P-C.; Yu, D.-Q.; He, C.-H.; Shen, F.-L.; Yang, J.-J.; *Acta Acad. Med. Sin.*, **1989**, *11*, 322-325.

[47] Nakano, K.; Oose, Y.; Masuda, Y.; Kamada, H.; Takaishi, Y.; *Phytochemistry*, **1997**, *45 (2)*, 293-296.
[48] Shiojima, K.; Arai, Y.; Masuda, K.; Takase, Y.; Ageta, T.; Ageta, H.; *Chem. Pharm. Bull.*, **1992**, *40*, 1683.
[49] Li, K.; Duan, H.; Kawazoe, K.; Takaishi, Y.; *Phytochemistry*, **1996**, *42*, 9023.
[50] Shiojima, K.; Masuda, K.; Suzuki, H.; Lin, T.; Ooishi, Y.; Ageta, H.; *Chem. Pharm. Bull.*, **1995**, *43*, 1634.
[51] Seo, S.; Tomita, Y.; Tori, K.; *J. Chem. Soc., Chem. Commun.*, **1975**, 954-955.
[52] Tanaka, N.; Duan, H.; Takaishi, Y.; Kawazoe, K.; Goto, S.; *Phytochemistry*, **2002**, *61*, 93-98.
[53] Sotanaphun, U.; Suttisri, R.; Lipipun, V.; Bavovada, R.; *Chem. Pharm. Bull.*, **2000**, *48 (9)*, 1347-1349.
[54] Chávez, H.; Estévez-Braun, A.; Ravelo, A.G.; González, A.G.; *Tetrahedron*, **1997**, *53 (18)*, 6465-6472.
[55] Januario, A.H.; Das, M.F.; Da Silva, F.; Vieira, P.C.; Fernández, J.B.; *Phytochemistry*, **1992**, *31*, 1251-1253.
[56] Drewes, S.E.; Mashimbe, M.J.; Field, J.S.; Ramesar, N.; *Phytochemistry*, **1991**, *30*, 3490-3493.
[57] Dantanarayana, A.P.; Kumar, N.S.; Muthukuda, P.M.; Wareer, M.I.; *Phytochemistry*, **1982**, *21*, 2065.
[58] Mulholland, D.A.; Kotsos, M.; Mahomed, H.A.; Taylor, A.H.; *Phytochemistry*, **1998**, *49*, 2457-2460.
[59] Ankli, A.; Heilmann, J.; Heinrich, M.; Sticher, O.; *Phytochemistry*, **2000**, *54*, 531-537.
[60] Alvarenga, N.; Ferro, E.A.; Ravelo, A.G.; Kennedy, M.L.; Maestro, M. A.; González, A.G.; *Tetrahedron*, **2000**, *56*, 3771-3774.
[61] Ferth, R.; Kopp, B.; *Pharmazie*, **1992**, *47*, 626-629.
[62] Kawaguchi, K.; Asaka, I.; Hirotani, M.; Furuya, T.; Katsuki, S.; *Phytochemistry*, **1993**, *34*, 1317-1321.
[63] Kopp, B.; Kubelka, W.; *Planta Med.*, **1982**, *45*, 195-202.
[64] Hanada, R.; Abe, F.; Möri, Y.; Yamauchi, T.; *Phytochemistry*, **1992**, *31*, 3547-3551.
[65] Ngassapa, O.D.; Soejarto, D.D.; Che, C-T.; Pezzuto, J.M.; Farnsworth, N.R.; *J. Nat. Prod.*, **1991**, *54*, 1353-1359.
[66] Johnson, A.W.; King, T.J.; Martin, R.J.; *J. Chem. Soc.*, **1961**, 4420-4425.
[67] Nakanishi, K.; *Comprehensive Natural Products Chemistry*, Vol.1, **1998**, p. xxxvii.
[68] González, A.G.; Bazzocchi, I. L.; Jimenez, I.A.; Moujir, L.; *Studies in Natural Products Chemistry.Atta-ur-Rahman,Ed.*, Elsevier Science. B.V.Amsterdan. **2000**, *23*, 649-738
[69] Chou, T.Q.; Mei, P.F.; *Chinese J. Physiol*, **1936**, *10*, 259
[70] Gisvold, O. J.; *Amer. Pharm. Assoc.*, **1939**, *28*, 440.
[71] Bhatnagar, S.S.; Divekar, P.V. J.; *Sci. Industr. Res*, **1951**, *10B*, 56.
[72] González, A.G.; Alvarenga, N.L.; Bazzocchi, I.L.; Ravelo, A.G.; Moujir, L.; *Planta Medica*, **1998**; *64*, 769 – 771.

[73] Alvarenga, N.L.; Velázquez, C.A.; Gómez, R.; Canela, N.J.; Bazzocchi, I.L.; Ferro, E.A.; *J. Nat. Prod*, **1999**, *62*, 750 – 751.
[74] González, A.G.; González, C.M.; Ferro, E.A.; Ravelo, A.G.; Domínguez, X.A.; *J.Chem. Res.*, **1988**, (S) *20*, (M) 273.
[75] González, A.G.; Francisco, C.G.; Freire, R.; Hernández, R.; Salazar, J.A.; Juarez, E.; *Phytochem*istry, **1977**, *32*, 282.
[76] Sneden, A.T.; *J. Nat. Prod.*, **1981**, *44*, 503.
[77] Figueiredo, J.N.; Räz, B.; Séquin, U.; *J. Nat. Prod.*, **1998**, *61*, 718 – 723.
[78] Brown, P.M.; Moir, M.; Thomson, R.H.; King, T.J.; Krishnamoorthy, V.; Seshadri, T.R.; *J. Chem. Soc., Perkin Trans. 1*, **1973**, 2721.
[79] Sotanaphun, U.; Suttisri, R.; Lipipun, V.; Bavovada R.; *Phytochemystry*, **1998**, *49*, 1749 – 1755.
[80] Martin, J.D.; *Tetrahedron*, **1973**, *29*, 2997.
[81] Chávez, H.; Valdivia, E.; Estévez-Braun, A.; Ravelo, A.G.; *Tetrahedron*, **1998**, *54*, 13579 – 13590.
[82] Gunaherath, G.M.K.B.; Gunatilaka A.A.L.; Sultanbawa, M.U.S.; Wazeer, M.I.M.; *Tetrahedron Lett,*, **1980**, *21*, 2025.
[83] Morota, T.; Yang, C-X.; Ogino, T.; Qin, W-Z.; Katsuhara, T.; Xu, L-H.; Komatsu, Y.; Miao, K-L.; Maruno, M.; Yang, B-H.; *Phytochemistry*, **1995**, *39 (5)*, 1159-1163.
[84] Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H.; Iitaka, Y.; *J Nat Prod*, **1994**, *57*, 1675.
[85] González, A.G.; Alvarenga, N.L.; Ravelo, A. G.; Jiménez, I. A.; Bazzocchi, I. L.; Canela, N.J.; Moujir, L.M.; *Phytochem*ystry, **1996**, *43*, 129 –132.
[86] Gamlath, C. B.; Gunatilaka, A.A.L.; *Phytochem*istry, **1988**, *27*, 3221.
[87] Dias, M. N.; Fernando, H. C.; Gunatilaka, A.A.L.; Tezuka, Y.; Kikuchi, T.J.; *J Chem Res*, **1990**, *(S)*, 238-239.
[88] Shirota, O.; Morita, H.; Takeya, K.; Itokawa, I.; *Chem. Pharm. Bull.*, **1998**, *46 (1)*, 102-106.
[89] González, A.G.; Alvarenga, N.L.; Estévez-Braun, A.; Ravelo, A.G.; Bazzocchi, I.L.; Moujir, L.M.; *Tetrahedron*, **1996**, *52*, 9597-9608.
[90] Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H.; *J. Nat. Prod.*, **1997**, *60*, 1100-1104.
[91] González, A.G.; Kennedy, M.L.; Rodríguez, F. M.; Bazzocchi, I.L.; Jiménez, I.A.; Ravelo, A.G.; Moujir, L.; *Tetrahedron*, **2001**, *57*, 1283-1287.
[92] Juling, G.; Shixiang, Y.; Xichun, H.; Shixi, X.; Dada, L.; *Chinese Medical Journal*, **1981**, *94*, 405.
[93] Kita, M.; Ohmoto, Y.; Yamaguchi, N.; Imanishi, J.; *Microbiology and Inmunology*, **1992**, *36*, 507.
[94] Ghezzi, P.; Dinarello, C.A.; *J. Inmunolog.*, **1988**, *140*, 4238.
[95] Miyazaki, N.; Sato, K.; Goto, M.; Sasano, M.; Natsuyama, M.; Inoue, K.; Nishioka, K.; *Arthritis and Reumatism*, **1988**, *31*, 480.
[96] Shirota, O.; Tamemura, T.; Morita, H.; Takeya, K.; Itokawa, H.; *J. Nat. Prod.*, **1996**, *59 (11)*, 1072.
[97] Chávez, H.; Estévez-Braun, A.; Ravelo, A.G.; González, A.G.; *Tetrahedron*, **1997**, *53*, 6465-6472.

[98] Chávez, H.; Estévez-Braun, A.; Ravelo, A.G.; González, A.G.; *J. Nat. Prod.*, **1998**, *61*, 82-85.

[99] González, A.G.; Ravelo, A.G.; Bazzocchi, I.L.; Jiménez, J.; Gomzález, C.M.; Luis, J.G.; Ferro, E.A.; Gutiérrez, A.; Moujir, L.; Las Heras, F.G.; *Il Fármaco*, **1988**, *43*, 501-505.

[100] Takasaki, M.; Konoshoma, T.; Fujitani, K.; Yoshida, S.; Nishimura, H.; Tokuda, H.; Nishino, H.; Iwashima, A.; Kozuka, M.; *Chem. Pharm. Bull.*, **1990**, *38*, 2737.

[101] Ohigashi, Y.; Takamura, H.; Koshimizu, K.; Tokuda, H.; Ito, Y.; *Cancer Letters*, **1986**, *30*, 143.

[102] Avilla, J.; Teixidó, A.; Velázquez, C.; Alvarenga, N.; Ferro, E.; Canela, R.; *J. Agric. Food Chem.*, **2000**, *48*, 88-92.

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# BIOLOGICALLY ACTIVE SUBSTANCES FROM THE GENUS *BACCHARIS* L. (COMPOSITAE)

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**ABSTRACT:** Traditional herbal remedies have increased in popularity in recent years. The plant family Asteraceae (Compositae) comprises a large number of species that have been and are still used as medicinal plants, particularly in folk medicine. *Baccharis* is a New World genus belonging to the Compositae family consisting of more than 400 species, about 90% of which are located in South America. Many of these species have been used since ancient times as folk remedies for some treatment purposes (reducing phlegm, relieving cough, invigorating blood circulation, stopping pain, inducing diuresis, as an anthelmintic, anti-inflammatory, stomachic agent,...). According to the literature, over 100 *Baccharis* species have been investigated to reveal that they contain many classes of secondary metabolites. The most widespread compounds reported are clerodane and labdane diterpenoids as well as triterpenoids of the oleanane series. In addition, kaurane diterpenoids, cinnamic acid esters, coumarin derivatives and flavonoids are common secondary metabolites. During the intensive investigation of the chemical components of the *Baccharis* genus, much attention has been paid to its bioactive constituents. Their occurrence in the genus *Baccharis*, as well as other constituents, and the chemical and biological relationships between them, have made them the subject of the present review. This chapter deals principally with bioactive constituents characterized from the *Baccharis* species in order to obtain a better understanding of the biological significance of this large genus.

## INTRODUCTION

Medicinal plants are an important element of indigenous medical systems in most countries. These resources are usually regarded as part of a culture's traditional knowledge. For many years, Europe has benefitted from exchange with other continents, and many of the pure natural products and some of the phytotherapeutic preparations used today are derived from plants used in

indigenous cultures. The role of the ethnobotanist in the search for new drugs was of continuous importance until the second half of the 20$^{th}$ century, when other approaches became more "fashionable" [1].

The plant family Asteraceae (Compositae) comprises a large number of species that have been and are still used as medicinal plants, particularly in folk medicine. *Baccharis* L. is a New World genus belonging to the Compositae family, consisting of more than 400 species, about 90% of which are located in South America. Five are endemic to North America and Northern Mexico; 38 others occur in Mexico and continental Central America, many of these with part of their range in the United States; and an additional six or seven endemic species occur in the West Indies [2]. Most of them are used empirically by rural and indigenous populations, commonly as infusions or decoctions, for medicinal purposes or, to a lesser extent, as food or as raw material for different local industries. Due to the increasing interest in natural products, the urban use of some folk medicinal plants as herbal remedies is also increasing. A number of studies have been carried out on the biological activities of *Baccharis*, plants as well as on the identification of the active compounds. This review concerns the traditional use, scientific knowledge and major chemical constituents of several of the most commonly used medicinal plants belonging to the genus *Baccharis*.

## TRADITIONAL USES

*Baccharis* is a large, strictly American genus, with rather diverse uses [3,4]. Some of the traditional uses of the principal medicinal species are discussed below.

*Baccharis glutinosa* (B. & P.) Pers. ("Jarilla") is used in several states of Mexico for gynecological and digestive disorders, as well as skin diseases. Branches in aguardiente (an alcoholic beverage) are taken orally for gynecological problems. The mashed leaves are used to clean wounds and treat scalp diseases. Tea made from the leaves is taken to lose weight and as a contraceptive [5].

*Baccharis conferta* (H.B.K.) ("Quauhizquiztli") is employed in the region of Veracruz, Mexico, to treat stomach aches. It is also recommended for

strengthening mothers after birth, for using as a laxative, for stimulating urination and for topically treating baldness and insect bites [6,7].

*Baccharis salicifolia* (R. et P.) Pers. ("T'ola alta", "Chilca marga"): the green leaves/leafy branches are applied externally as a remedy for inflammation, diarrhoea and dysentery [8].

*Baccharis sarothroides* A. Gray ("Desert broom"): a tea made by boiling the twigs is taken as a remedy for colds. The same tea is rubbed on muscles for relief [8].

*Baccharis serraefolia* D. C. ("Tzajal") is a popular form of treatment for various gastrointestinal illnesses with the Tzeltal and Tzotzil of Chiapas, Mexico [7]. Ethnomedical information shows that this plant has been widely used to treat diarrhoea by the past and present Mayas [9].

*Baccharis multiflora* Bent. ("Quappatli", "Malinalli"): an infusion prepared from the leaves is said to be effective against catarrhs and was used for urinary problems in nineteenth century Mexico [8].

*Baccharis trinervis* (Lam) Pers. ("Chilca") is employed in the treatment of high fever, edema, sores and muscle cramps. It is also applied in the case of dizziness and lack of blood. In the Veracruz region of Mexico, a preparation made from the leaves is used for the treatment of typhoid fever and as a remedy for gastrointestinal disorders [10,11].

*Baccharis vaccinoides* H.B.K. ("Chilco") is one of the most popular remedies from the Chiapas region of Mexico for gastrointestinal disorders [11].

*Baccharis grisebachii* Hieron ("T'ola alta", "Quinchamal"), *Baccharis incarum* Wedd. ("Lejia") and *Baccharis latifolia* Pers. ("Chilca") are used in Argentina as decoctions or poultices of leaves for wound healing [12,13].

*Baccharis coridifolia* D. C. ("Mio-mio", "Romerillo") is used externally as an anti-inflammatory. The vapours along with sulphur are used to treat horse's distemper and for the treatment of horse's external parasites [8].

*Baccharis tucumanensis* (H. et A.) ("Suncho blanco", "Chilca") is used in several regions of Argentina as an abortive [13].

*Baccharis notosergilla* Grises. ("Carqueja") is used in the folk medicine of the Northeast of Argentina, Uruguay and Paraguay as a diuretic and digestive agent [13].

*Baccharis floribunda* H.B.K. ("Niquitao") and *Baccharis tricuneata* (L.f.) Pers. ("Sanalotodo") are used in Venezuela as decoctions or infusions of leaves and stems for skin infections and diabetes [14,15].

*Baccharis genistelloides* Lam. = *Baccharis trimera* (Lam.) Pers. ("Carqueja", "Charara") is used for liver disorders and as an antithermic. Other popular uses in Brazil include digestive disorders, malaria, diabetes, ulcers, sore throat and tonsilitis, anaemia, diarrhoea, indigestion, urinary inflammation, intestinal worms and leprosy [16,17].

*Baccharis gaudichaudiana* D. C. ("Chilca melosa") and *Baccharis articulata* (Lam.) Pers. ("Carquejilla") are used traditionally as decoctions or infusions as an antidiabetic remedy in Paraguay [13].

*Baccharis rubricaulis* Rubby ("Chilca") is used by the rural and indigenous populations of Bolivia for treating different types of mucous ailments [18].

## CHEMISTRY

To date more than 100 species from the large genus *Baccharis* have been investigated chemically. The most widespread compounds are clerodane diterpene derivatives, but labdane derivatives have also been isolated. Furthermore, typical acetylenic compounds, baccharis oxide, a unique triterpene, and derivatives of hydroxyacetophenone are present, although it is mainly other biologically active constituents, e.g. flavonoids, sesquiterpenes and essential oils that have been reported in the last years. The constituents of *Baccharis* species compiled from different sources are presented below.

## TERPENOIDS

Terpenoids occur in abundance in the plant world where they possess many different kinds of functions. Essentially, they are derived from the basic 5-carbon isoprene unit, biosynthetically as isopentenyl pyrophosphate, which is itself derived from acetate via mevalonic acid. They may be classified into diverse groups according to the number of isoprene units, e.g., monoterpenes ($C_{10}$), sesquiterpenes ($C_{15}$), diterpenes ($C_{20}$), triterpenes and steroids ($C_{30}$),...

## Diterpenoids

The diterpenoids are a large and ubiquitous family of isoprenoids derived from *2E,6E,10E*-geranylgeranyl pyrophosphate. Of all the families of natural products, the diterpenoids have one of the widest ranges of biological activity. The clearest classification of the cyclic diterpenoids (abietane, labdane, clerodane,...) is based on biogenetic considerations. The variety of structural types found amongst the diterpenoids has led to their use as phytochemical markers. Indeed, many of the diterpenoids found in recent years have been isolated in the course of phytochemical surveys of the Compositae.

Characteristic constituents from the *Baccharis* genus, one member of the Compositae, are the *neo*-clerodane type diterpenes. For example, the aerial parts of *B. trinervis*, a representative of which is used in South American folk medicine, furnished 24 *neo*-clerodane type diterpenes, many of them new, e.g., methyltrineracetal, Fig. **(1)** and epi-methyltrineracetal, Fig. **(2)** [19].

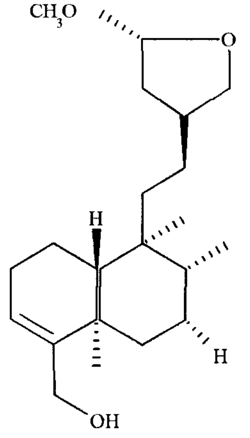


**Fig. (1).** Structure of methyltrineracetal

The investigation of the aerial parts of nine *Baccharis* species from Argentina gave seven new *ent*-clerodane derivatives from *B. salicifolia* [20,21].

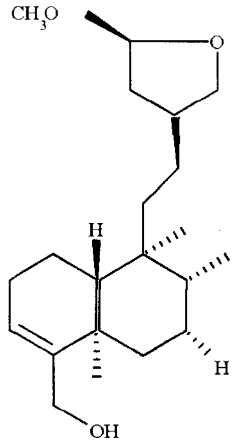


**Fig. (2).** Structure of epi-methyltrineracetal

From another Argentinian *Baccharis* species, *B. articulata*, three diterpenes of *neo*-clerodane type, articulin, Fig. **(3)**, articulin acetate and barticulidiol, Fig. **(4)**, together with the known bacchotricuneatin A, Fig. **(5)**, were isolated [22,23].

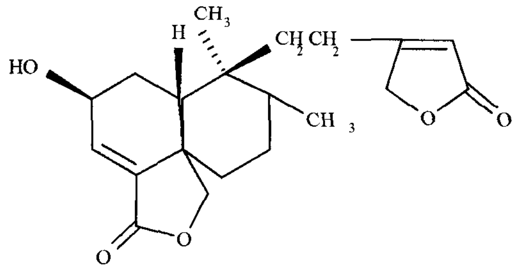


**Fig. (3).** Structure of articulin

This diterpene lactone, bacchotricuneatin A was also isolated from *B. tricuneata* [24] and *Baccharis cassinaefolia* D. C. [25].

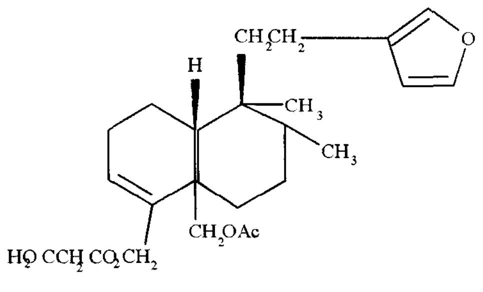


**Fig. (4).** Structure of barticulidiol

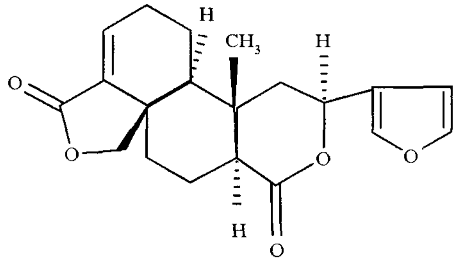


**Fig. (5).** Structure of bacchotricuneatin A

Li *et al.* [26] also reported the isolation and structure elucidation of a new clerodane diterpenoid from *Baccharis myrsinites* (Lam) Pers., a species endemic to the Dominican Republic, while the aerial parts of *Baccharis santelicis* Phi., an endemic species from Chile, afforded several known furoclerodanes [27]. From another endemic species from Chile, *B. incarum*, a new *neo*-clerodane diterpenoid named bincatriol, Fig. (6) was isolated [28].

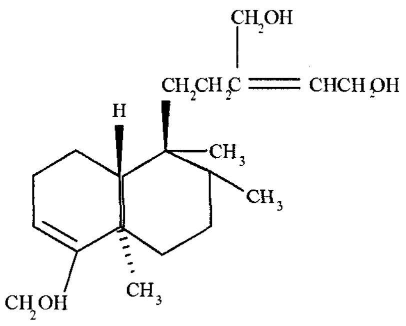


**Fig. (6).** Structure of bincatriol

Gambarro *et al.* [29] reported the isolation and characterization of four clerodane derivatives, two of them new: hardwickiic acid, Fig. (7) and hautriwaic acid, Fig. (**8**) present in *Baccharis macraei* Hook et Am, a shrub that grows in the coastal area of Central Chile, while *Baccharis paniculata* D. C. from Northem Chile also yielded two new *neo*-clerodane diterpenoids [30].

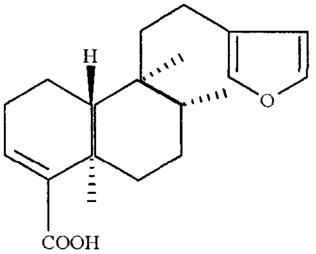


**Fig. (7).** Structure of hardwickiic acid

These diterpenoids, hautriwaic acid and its derivative 2β-hydroxyhautriwaic acid which is a novel natural product, were also isolated from the leaf resin of *B. sarothroides* and *B. vaccinoides* [31].

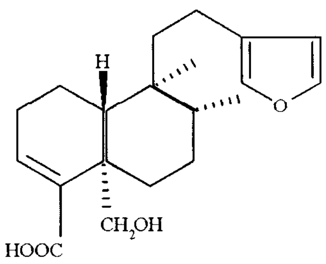


**Fig. (8).** Structure of hautriwaic acid

Morales *et al.* [32] isolated another compound of this type, a new *ent*-clerodane furanoditerpenoid named bolivianol, Fig. **(9)** from *Baccharis boliviensis* (Wedd.) Cuatr. *B. boliviensis*, widely distributed from Peru across Bolivia to Chile and Argentina, also afforded known *ent*-clerodane derivatives [33].

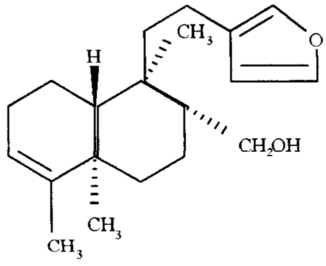


**Fig. (9).** Structure of bolivianol

Jakupovic *et al.* [34] investigated some representatives of the genus *Baccharis* which grow in Texas and Mexico, and seven new *ent*-clerodane diterpenoids have been described from *Baccharis pteronioides* D. C. Novel *neo*-clerodane diterpenoids were also isolated from the aerial parts of *B. genistelloides*, which were collected in the area of Sao Paulo and neighbouring states of Brazil [35-37] and from *Baccharis scoparia* (L.) Sw., a species widespread in the Caribbean Islands [38].

The most widespread diterpenes reported in *Baccharis* genus are clerodanes, but labdane- and kaurane-type, and to a lesser extent beyerane-type diterpenoids have also been isolated. The investigation of ten *Baccharis* species afforded four new *ent*-labdane diterpenes from *Baccharis oxydonta*

D. C. [39] and a new labdane aldehyde, Fig. **(10)** from *B. scoparia*, a species widespread in the Caribbean Islands [38].

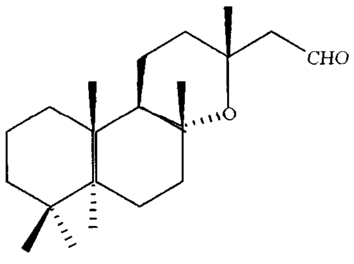


**Fig. (10).** Structure of a new labdane aldehyde isolated from *Baccharis scoparia*

From the aerial parts of *Baccharis pingraea* D. C. collected in Chile, several known furolabdane diterpenoids, together with two novel diterpenes, furolabda-6,8-dien-17-oic acid, Fig. **(11)** and furolabda-7-en-17-oic acid, Fig. **(12)** were isolated [20,40,41].

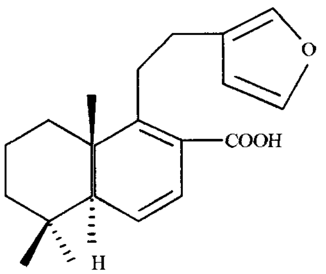


**Fig. (11).** Structure of furolabda-6,8-dien-17-oic acid

New labdane-type diterpenes were also reported from *B. boliviensis* [33], *Baccharis medullosa* D. C. [42], *B. paniculata* [43] and *Baccharis sternbergiana* Stend. [44]. As we can see from the data reported in the literature, in one species clerodanre diterpenes and in the other labdanes were present, indicating that there are chemically different groups in the large genus *Baccharis*.

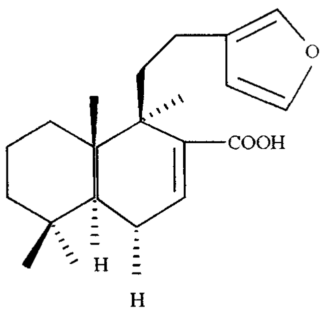


**Fig. (12).** Structure of furolabda-7-en-17-oic acid

From the kaurane-type diterpenoids, *ent*-kaurenic acid, Fig. **(13)** and *ent*-kaurenol, Fig. **(14)** were reported from the roots of *Baccharis ramoisissima* Gardn. [39], *Baccharis minutiflora* Mart. [45] and *Baccharis intermixta* Gardn. [25].

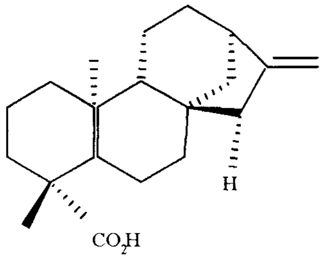


**Fig. (13).** Structure of *ent*-kaurenic acid

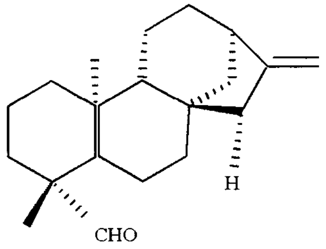


**Fig. (14).** Structure of *ent*-kaurenol

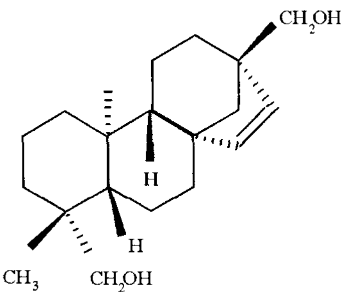


**Fig. (15).** Structure of *ent*-beyer-15-en-17,19-ol

Furthermore, from the species *Baccharis tola* Phil. growing in Chile, new *ent*-beyerane-type diterpenoids were reported, such as *ent*-beyer-15-en-17,19-ol, Fig. **(15)** and *ent*-4α-hydroxy-19-norbeyer-15-ene, Fig. **(16)** [46].

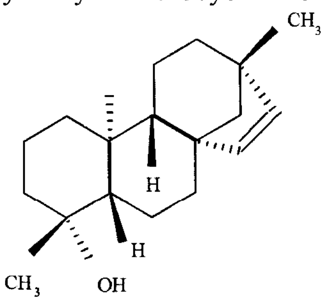


**Fig. (16).** Structure of *ent*-4α-hydroxy-19-norbeyer-15-ene

## Sesquiterpenes

As can be seen from the last paragraph, the most usual type of secondary metabolites isolated from the genus *Baccharis* are clerodane-type diterpenes. However, other types of terpenes are also widespread in this genus; sesquiterpenes with different kinds of skeletons being one of them. The sesquiterpenoids are a widespread group of substances occurring in many different organisms, and these compounds form the largest class of terpenoids. More than 100 sesquiterpenoid skeleta are known, and several thousands of compounds of this class have been isolated and identified. The

sesquiterpenoids are formed by the condensation of isopentenyl pyrophosphate with 3,3-dimethylallyl pyrophosphate to give geranyl pyrophosphate. Further addition of a molecule of isopentenyl pyrophosphate forms *2E,6E*-farnesyl pyrophosphate, which has the 15 carbon atoms characteristic of sesquiterpenoids.

Reports on the sesquiterpene composition of *Baccharis* genus are recorded in the literature. From some representatives of this genus growing in Texas and Mexico, sesquiterpene hydrocarbons, such as germacrane D and caryophyllenepoxide were isolated [34]. From *Baccharis dracunculifolia* D. C. sesquiterpene hydrocarbons like germacrane D, δ-cadinene, caryophyllene, ledene and sesquiterpene alcohols such as nerolidol and spathulenol were also identified [47]. Norte *et al.* [48] reported the isolation and chemical characterization of a sesquiterpene possessing a new regular carbon skeleton, called spartidienedione, Fig. **(17)** which was obtained from *Baccharis spartioides* (Hook et Arn) Remy. collected in Northern Chile.

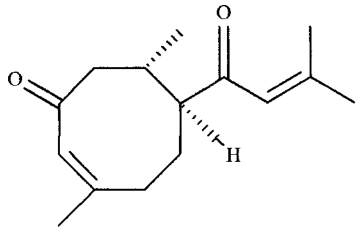


**Fig. (17).** Structure of spartidienedione

A new amorphane sesquiterpene γ-lactol derivative, O-methylbaccharocephol, Fig. **(18)** was isolated from *Baccharis sphaerocephala* B&N [49]. This compound possesses a very rare type of carbon skeleton, with two *cis*-fused six-membered rings, a five-membered-ring γ-lactone and an epoxide. Chemical analysis of *Baccharis salicina* Torr & Gray afforded two new guaianolides, bacchariolides A, Fig. **(19)** and B, Fig. **(20)** [50].

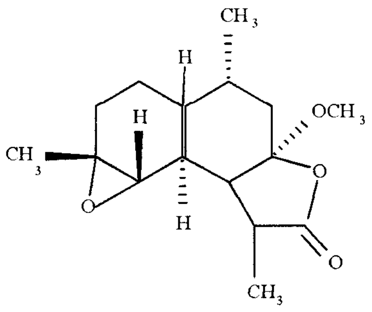


**Fig. (18).** Structure of O-methylbaccharocephol

Additionally, the aerial parts of *Baccharis petiolata* D. C. from a collection in Chile afforded an unusual dimeric sesquiterpene [27], while *B. boliviensis*, a species widely distributed from Peru across Bolivia to Chile and Argentina, also yielded new sesquiterpenes [33].

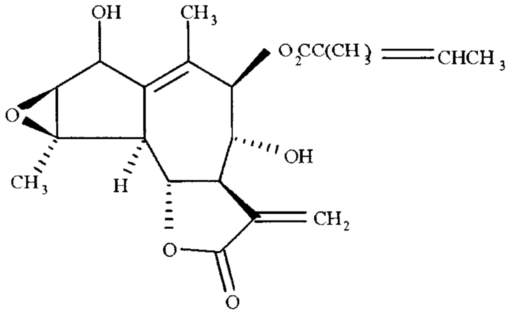


**Fig. (19).** Structure of bacchariolide A

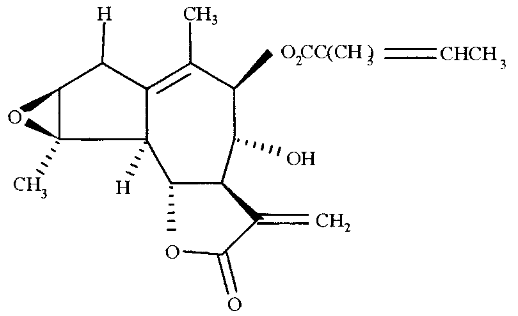

**Fig. (20).** Structure of bacchariolide B

Trichothecenes are a family of sesquiterpenoid compounds produced by various genera of fungi. However, several years ago a Brazilian shrub, *Baccharis megapotamica* Spreng., was reported to contain a series of potent cytotoxic agents belonging to the trichothecene complex. This was surprising as the trichothecene mycotoxins had previously been found to be produced only by soil fungi, e.g. *Fusarium* and *Myrothecium*. There are two groups of trichothecenes: simple and macrocyclic, depending on the presence or absence of a cyclic linkage between C-4 and C-15. Several macrocyclic trichothecenes, such as roridins, verrucarins and baccharinoids have been isolated from *B. megapotamica* and another Brazilian *Baccharis* species, *B. coridifolia* [51-53].

## Triterpenoids

The triterpenoids constitute a large diverse group of active natural products derived from squalene. In excess of 4000 triterpenoids have been isolated so far and more than 40 skeletal types have been identified. In general, triterpenes arise by cyclisation of squalene or the *3S* isomer of 2,3-epoxy-2,3-dihydrosqualene. Cyclisation of this isomer leads to 3β-hydroxytriterpenoids which, by oxidation and reduction, can be transformed into 3α-hydroxyterpenoids.

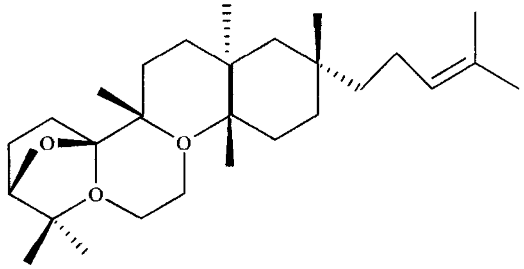


**Fig. (21).** Structure of baccharis oxide

Reports on the triterpene composition of *Baccharis* genus are recorded in the literature. Baccharis oxide, Fig. **(21)** is a common and unique triterpene found in many *Baccharis* species, such as *Baccharis halimifolia* L. [54] and *B. salicifolia* [55]. The NMR spectra of this compound was assigned ambiguously [56], but recently Nurnberg *et al.* [57] established the total NMR chemical shift assignments of baccharis oxide.

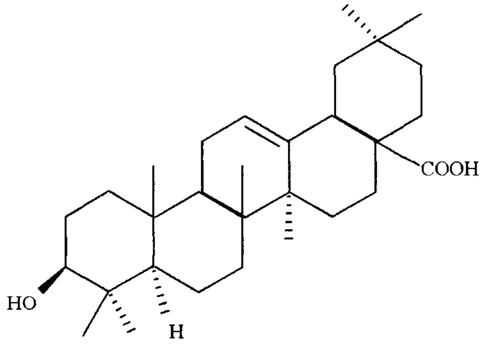


**Fig. (22).** Structure of oleanolic acid

The most widespread compounds of this group reported in the genus *Baccharis* were oleanolic triterpenoids. For example, oleanolic acid, Fig. **(22)** and maniladiol, Fig. **(23)** were reported from *B. myrsinites*, a species endemic to the Dominican Republic [26] and *Baccharis heterophylla* H.B.K. collected in Texas and Mexico [34].

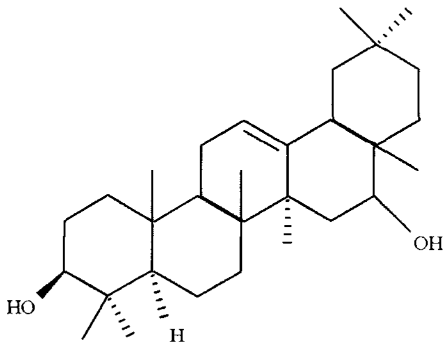


**Fig. (23).** Structure of maniladiol

In several Chilean *Baccharis* species such as *Baccharis linearis* (R. et Pav.) Pers., *Baccharis rhomboidalis* J. Remy and *Baccharis solieri* D. C., oleanolic acid, Fig. **(22)**, stigmasterol, β-amyrin, Fig. **(24)** and friedelinol were present [58].

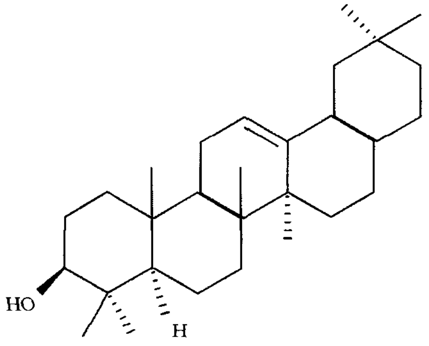


**Fig. (24).** Structure of β-amyrin

From *B. tricuneata* collected in the surroundings of Santafe de Bogota (Colombia), several triterpenoids, e.g. β-sitosterol, α-amyrin acetate, friedelin, Fig. **(25)** and ursolic acid, Fig. **(26)** were also reported [59], while the species *B. coridifolia* yielded schottenol glucoside, Fig. **(27)**, uvaol, Fig. **(28)** and ursolic acid, Fig. **(26)** [60].

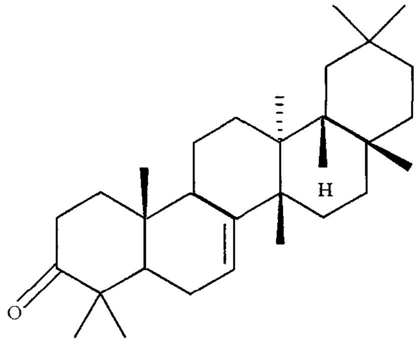


**Fig. (25).** Structure of friedelin

Furthermore, the investigations of *B. serraefolia* and *B. genistelloides* afforded phytol, β-sitosterol and stigmasterol as triterpenoid compounds [61,62].

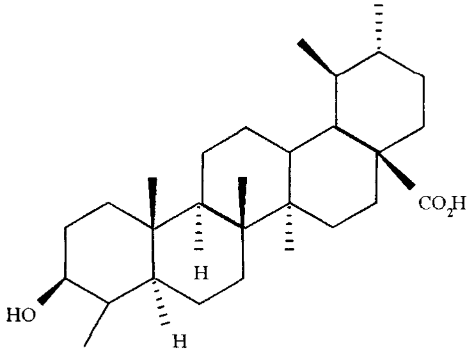


**Fig. (26).** Structure of ursolic acid

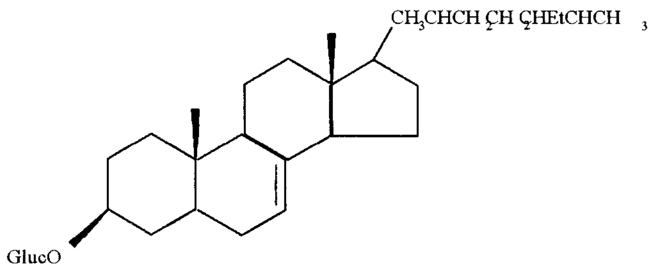


**Fig. (27).** Structure of schottenol glucoside

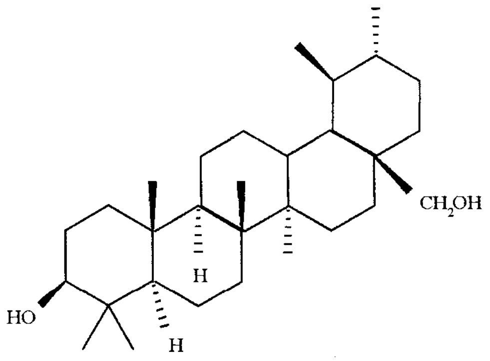


**Fig. (28).** Structure of uvaol

## Essential Oils

Volatile or essential oils, as their name implies, are volatile in steam and are generally complex mixtures of hydrocarbons and oxygenated compounds derived from these hydrocarbons. The odour and taste of volatile oils is mainly determined by these oxygenated constituents. In chemical structure, most essential oils are terpenoid in origin.

The strong aromatic odour of some species of *Baccharis* genus is due mainly to high concentrations of volatile terpenes, constituents of their essential oils, specially in leaves and flowers. Essential oils from the genus *Baccharis* have been studied in several species from South America. More than 100 constituents were identified in a study of the composition of the essential oil of Brazilian Vassoura oil (*B. dracunculifolia*) [63]. The oil was composed of 19% monoterpene hydrocarbons, 2% oxygenated monoterpenes, 38% sesquiterpene hydrocarbons and 35% oxygenated sesquiterpenes, e.g. 15% nerolidol, Fig. **(29)**. The essential oil from this species *B. dracunculifolia*, harvested in Uruguay, was also analyzed by Loayza *et al.* [64], together with the oils of *B. latifolia* and *B. salicifolia* harvested in the region of Cochabamba (Bolivia). In the three species, the concentration of germacrone (the main compound) is in the 20-30% range

depending on the harvesting period. A variety of monoterpenes (30%) and sesquiterpenes (15%) are also present.

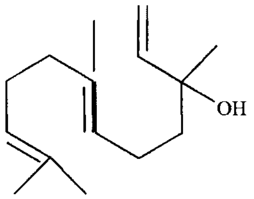


**Fig. (29).** Structure of nerolidol

Samples of the essential oil from one of these species, *B. salicifolia* collected in the province of Cordoba (Argentina), together with *Baccharis crispa* Spreng. and *Baccharis cordobensis* Heering were examined [65,66]. The major constituents were nerolidol (15.8%), Fig. **(29)**, cadinol (14.7%), Fig. **(30)** and cubenol (8.8%) from *B. cordobensis*, cadinol (9.4%), Fig. **(30)** and germacrane D (8.8%), Fig. **(31)** from *B. salicifolia* and nerolidol (27%), Fig. **(29)** from *B. crispa*.

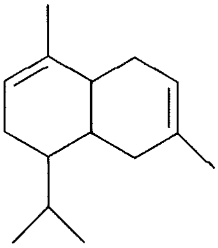

**Fig. (30).** Structure of cadinol

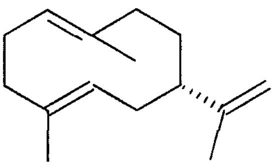

**Fig. (31).** Structure of germacrane D

Other endemic species from Argentina such as *B. articulata*, *Baccharis myrtilloides* (Mart.) Pax and *Baccharis rufescens* Spreng. found in the region of Traslasierra Valley were also investigated [67]. The major constituents of their essential oils were: for *B. articulata* β-caryophyllene (16.8%), Fig. **(32)** and nerolidol (15,6%), Fig. **(29)**; for *B. myrtilloides* germacrane D (9.5%), Fig. **(31)**; and for *B. rufescens* limonene (14.4%), Fig. **(33)** and nerolidol (14.5%), Fig. **(29)**.

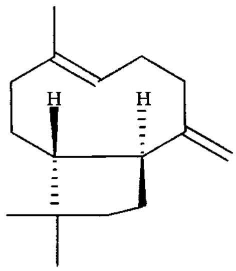


**Fig. (32).** Structure of β-caryophyllene

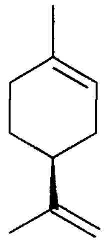

**Fig. (33).** Structure of limonene

Although the main component may vary, the sesquiterpene fraction is usually the dominant one. However, the essential oil of the aerial parts of *B. spartioides* from different Patagonian populations in Argentina consisted mainly of hydrocarbons and oxygenated monoterpenes [68].

More recently, Frizzo *et al.* [69] examined the essential oil from *Baccharis uncinella* D. C., popularly called "Vassoura lageana", which grows wild in the South of Brazil. No previous phytochemical studies have been reported concerning the composition of volatile compounds from this *Baccharis* species. The monoterpene profile is similar to those reported for other *Baccharis*, such as *B. dracunculifolia* and *B. crispa*, while the sesquiterpene

alcohol fraction shows nerolidol, Fig. **(29)**, ledol, viridiflorol, globulol and spathulenol, Fig. **(34)**.

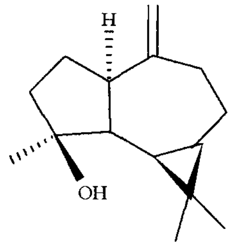


**Fig. (34).** Structure of spathulenol

## PHENOLIC COMPOUNDS

Phenols probably constitute the largest group of plant secondary metabolites. Widespread in nature, and found in most classes of natural compounds having aromatic moieties, they range from simple structures with one aromatic ring to highly complex polymeric substances. Phenols are important constituents of some medicinal plants and in the food industry they are used as colouring agents, flavourings, aromatizers and antioxidants. This section mainly deals with those phenolic classes of pharmaceutical interest found in the *Baccharis* genus, namely flavone and related flavonoid glycosides, coumarins and derivatives, and simple phenolic compounds.

### Flavonoids

The flavonoids, which occur both in the free state and as glycosides, are the largest group of naturally occurring phenols. They comprise a large number of natural substances, widespread in the plant kingdom, and found practically in all parts of the plants. More than 2000 of these compounds are now known, with nearly 500 occurring in the free state. They are formed from three acetate units and a phenylpropane unit, and are typed according to the state of oxygenation of the $C_3$ unit.

A survey of current available chemical data suggests that methylated flavones and, to a lesser extent flavonols, mainly aglycones, are the main classes of phenolic compounds in *Baccharis* spp. This genus is well-known for producing flavones such as apigenin, Fig. **(35)** a simple flavone isolated from *B. tola* [46].

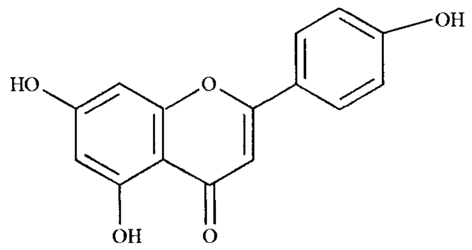


**Fig. (35).** Structure of apigenin

Several methylated derivatives of apigenin were also isolated from *B. trinervis*, together with two new flavones named pectolinaringenin (5,7-dihydroxy-6,4'-dimethoxyflavone), Fig. **(36)** and salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone), Fig. **(37)**, and one flavonol, pedunletin (5,4'-dihydroxy-3,6,7-trimethoxyflavone), Fig. **(38)** [70,71]. Some of these compounds, such as pectolinaringenin, Fig. **(36)**, together with the flavonoids nevadensin and demethoxysudachitin were also isolated from *B. grisebachii* [72].

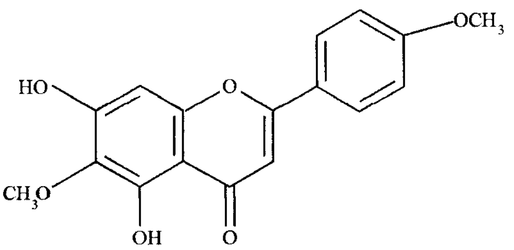


**Fig. (36).** Structure of pectolinaringenin

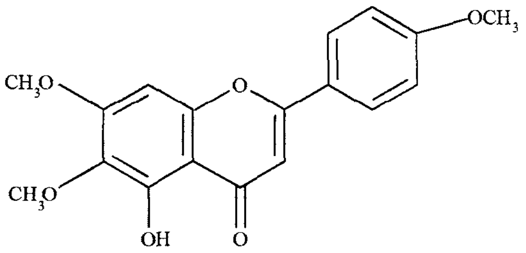


**Fig. (37).** Structure of salvigenin

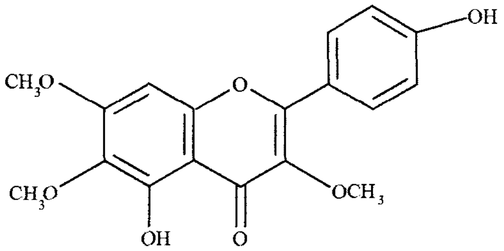


**Fig. (38).** Structure of penduletin

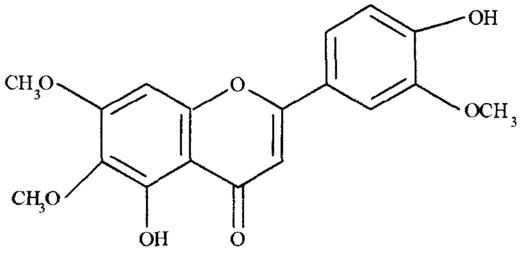


**Fig. (39).** Structure of eupatrin

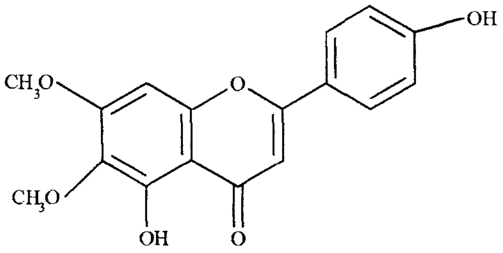


**Fig. (40).** Structure of cirsimaritin

In an investigation of the chemical constituents of *B. genistelloides*, four flavones named eupatrin, Fig. **(39)**, cirsimaritin, Fig. **(40)**, cirsiliol, Fig. **(41)** and genkwanin, Fig. **(42)**, together with the known apigenin, Fig. **(35)** were isolated [35,36,73].

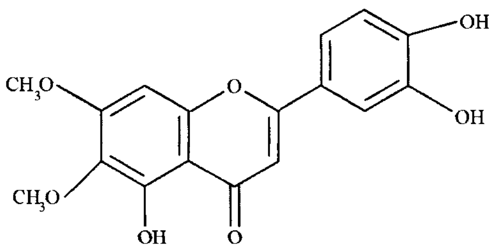


**Fig. (41).** Structure of cirsiliol

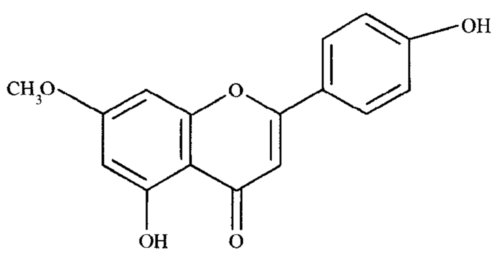


**Fig. (42).** Structure of genkwanin

The most widespread flavonoids reported in the *Baccharis* genus are flavones, but flavonols and flavanones have also been isolated. For example, Kupchan and Baverschmidt [74] reported the isolation and identification of two methylated flavonols, 3,4'-dimethoxy-3',5,7-trihydroxyflavone, Fig. **(43)** and centaureidin, Fig. **(44)** from *B. sarothroides*.

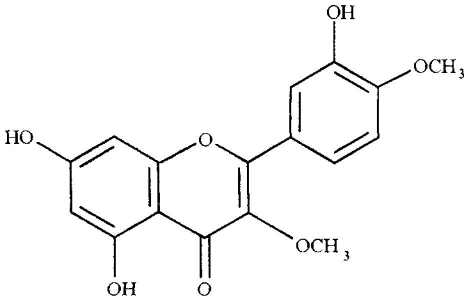


**Fig. (43).** Structure of 3,4'-dimethoxy-3',5,7-trihydroxyflavone

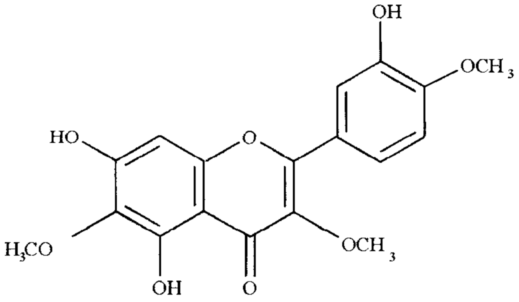


**Fig. (44).** Structure of centaureidin

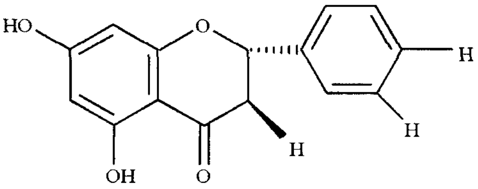


**Fig. (45).** Structure of pinocembrin

From the roots of *B. oxydonta*, Bohlmann *et al.* [39] isolated the flavanones pinocembrin, Fig. **(45)** and pinobanksin, Fig. **(46)**, while the aerial parts of *Baccharis varians* Gardn. afforded two 3-acetoxyflavanones, Fig. **(47)** [25].

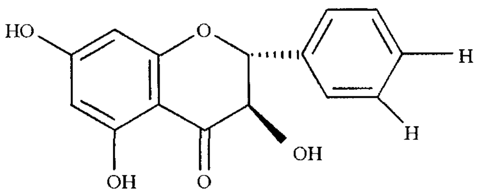


**Fig. (46).** Structure of pinobanksin

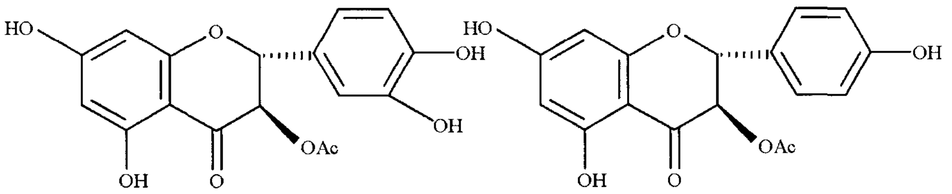


**Fig. (47).** Structure of 3-acetoxyflavanones isolated from *Baccharis varians*

## Coumarins

Derivatives of benzo-α-pyrone such as coumarin, Fig. **(48)** (the lactone of *o*-hydroxycinnamic acid) are common in plants both in the free state and as glycosides. Not all are phenolic but they are included here with the phenolic derivatives for convenience. Some 1000 natural coumarins have been isolated. Coumarin Fig. **(48)** itself has been found in about 150 species belonging to over 30 different families, although it is probably present in the undamaged plant as *trans-o*-glucosylcinnamic acid.

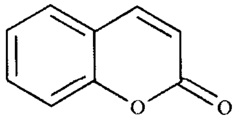


**Fig. (48).** Structure of coumarin

Reports on the coumarin composition from the *Baccharis* genus are recorded in the literature. For example, two long-chain esters of coumaric acid were isolated from *B. myrsinites*, a species endemic to the Dominican Republic [26]. During a chemosystematic investigation of the terpenic

composition in Argentinian *Baccharis* species, Zdero *et al.* [20] identified seven coumaric acid derivatives, Fig. **(49)**.

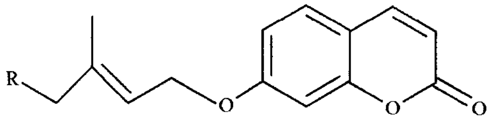


**Fig. (49).** Structure of coumaric acid derivatives isolated from Argentinian *Baccharis* spp.

From Bolivian *Baccharis* species, two prenylated coumaric acids, Fig. **(50)** were isolated from *B. boliviensis* [33], while *B. grisebachii*, an endemic species from Argentina, yielded 3-(3,3-dimethylallyl)-*p*-coumaric acid [72].

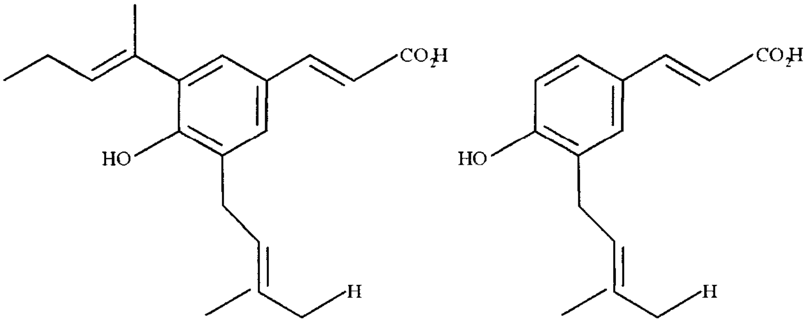


**Fig. (50).** Structure of prenylated coumaric acids isolated from *Baccharis boliviensis*

## Other Phenolic Compounds

In the genus *Baccharis* there are only reports of simple phenolic derivatives in *B. dracunculifolia*, an indigenous species throughout the Southeast parts of Latin America, which yielded ten new phenolic glycosides named dracunculifosides A-J, Fig. **(51)** [75,76].

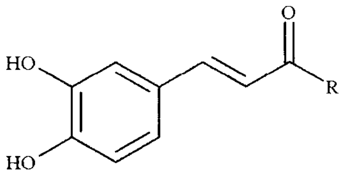


**Fig. (51).** Structure of dracunculifosides

## OTHER CHEMICAL CONSTITUENTS

Finally, reports of the occurrence of other different constituents in the *Baccharis* genus are available. In a research programme to investigate *Baccharis* species growing in different regions of Argentina a chemical investigation of *B. latifolia* and *Baccharis calvescens* D. C. afforded new *p*-hydroxyacetophenones, Fig. **(52)**, while *Baccharis magellamica* (Lam.) Pers. gave a propiophenone derivative, Fig. **(53)** [20,25,39]. Probably, these *p*-hydroxyacetophenones are of chemotaxonomic importance as the unusual position of the acetyl group seems to be very rare.

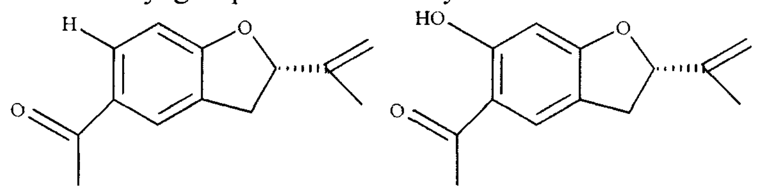


**Fig. (52).** Structure of new *p*-hydroxyacetophenones isolated from *Baccharis latifolia* and *Baccharis calvescens*

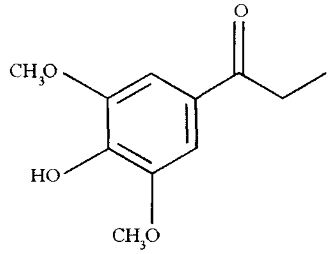


**Fig. (53).** Structure of a propiophenone derivative isolated from *Baccharis magellamica*

In a chemical investigation of *B. paniculata* from Northern Chile, two isomer acetylenic lactones were isolated [30], while the aerial parts of *B. salicifolia* yielded two prenyl derivatives, Fig. **(54)** [21].

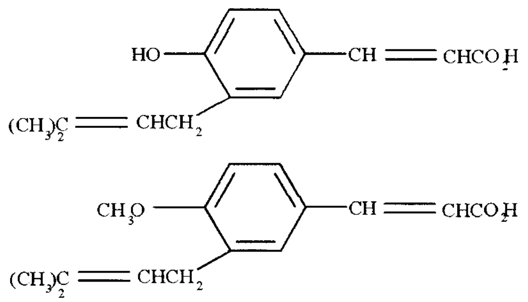


**Fig. (54).** Structure of prenyl derivatives isolated from *Baccharis salicifolia*

## PHARMACOLOGY

Compositae plants have been used for medicinal purposes for many centuries, showing different biological activities. Of these, plants belonging to the genus *Baccharis* are reported to have excellent medicinal value. Several pharmacological activities, including antitumoral, antimicrobial, antiviral and anti-inflammatory effects have been documented for extracts of *Baccharis* species and/or their constituents, and we present these below.

## ANTINEOPLASTIC ACTIVITY

Plants have been demonstrated to be a very viable source of clinically relevant anticancer compounds. However, ethnopharmacologic information has been poorly utilized in the past in the search for new principles against cancer. In many ethnomedical systems, reports of specific antitumoral uses of plants are rarely found, mainly because cancer is a disease that involves a complex set of signs and symptoms. A retrospective analysis of the National Cancer Institute programme showed that the percentage of active leads based on ethnomedicine was substantially above that based on taxonomy, which itself was higher than the active leads identified through random screening. Considering these observations, a set of ethnobotanical usages such as immune and skin diseases, inflammation, infections, parasitic and viral diseases, can be taken into account because they reflect disease states bearing

relevance to cancer or a cancer symptom [77]. Plants that are known for their toxic activity are also worth investigating.

Mongelli *et al.* [78] initiated a screening programme of Argentine medicinal plants for their antineoplastic activity. In this programme, two different criteria were used to choose the plant material: a group of plants with an ethnobotanical use related to cancer and a set of plants growing in the same region and randomly selected. In this work, 20 Argentine medicinal plants were examined using the brine shrimp bioassay, a assay with a good correlation with cytotoxicity in cancer cell lines and a variety of model tumour systems such as KB, P-388, L5178Y and L-1210. The most promising extracts were the dichloromethane extract of *B. coridifolia*, *B. grisebachii* and *B. Tucumanensis*. More recently, some of these active extracts were submitted to different bioassays in order to determine the cytotoxic activity: the potato disc and KB cells cytotoxicity assays and the DNA-methyl green assay for investigating the presence of substances that interact with DNA. The results obtained with *B. grisebachii* and *B. coridifolia* suggest that cytotoxicity could play an important role in the activities claimed for the plants under study [79,80].

As we can see in this review, a large number of diterpenoids and flavonoids, which are typical constituents of the *Baccharis* genus, were isolated from these species.These compounds might be partly or wholly responsible for the antitumour effects exhibited by the plants. Nakasugi and Komai [81] examined the antimutagen constituents from *B. genistelloides*, a Brazilian folk medicinal plant used to treat liver diseases and rheumatism. Four active substances were identified in the methanol extract: genkwanin, Fig. **(42)**, cirsimaritin, Fig. **(40)**, hispidulin and apigenin, Fig. **(35)**. It is thought that these flavones are the major active substances in this plant. As for the structure-activity relationships, a decreasing tendency of antimutagenic activity was found when the moiety of the hydroxy groups of flavones was replaced by the methoxy group. In the search for tumour inhibitors of plant origin, Kupchan and Bauerschmidt [74] found that alcoholic extracts of *B. sarothroides* showed significant inhibitory activity against cells derived from human carcinoma of the nasopharynx carried in cell culture, and reported the identification of two cytotoxic flavonols: 3,4'-dimethoxy-3',5,7-trihydroxyflavone, Fig. **(43)** and centaureidin, Fig. **(44)**.

In the terpenoid group, three labdane diterpenes, gaudichaudols A-C, Fig. **(55)**, six clerodane diterpenoids, gaudichaudones A-E, Fig. **(56)** and articulin acetate isolated from *B. gaudichaudiana* were evaluated in P-388 lymphocytic leukemia cells as well as a battery of human cancer cell lines [82,83]. Among them, gaudichaudol C, gaudichaudone A and articulin acetate exhibited significant cytotoxic activity against certain cancer cells.

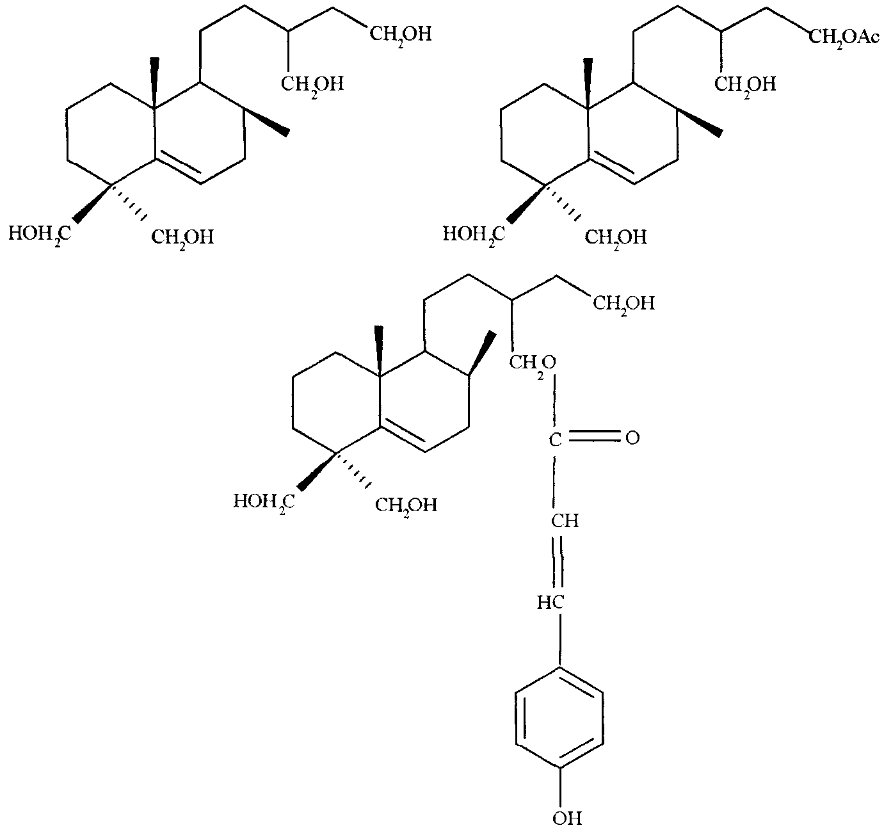


**Fig. (55).** Structure of gaudichaudols A-C

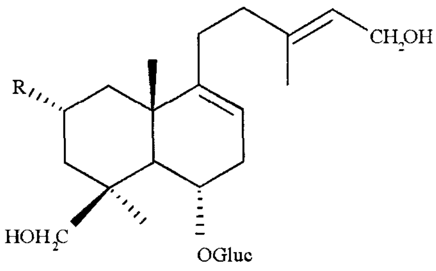


**Fig. (56).** Structure of gaudichaudones

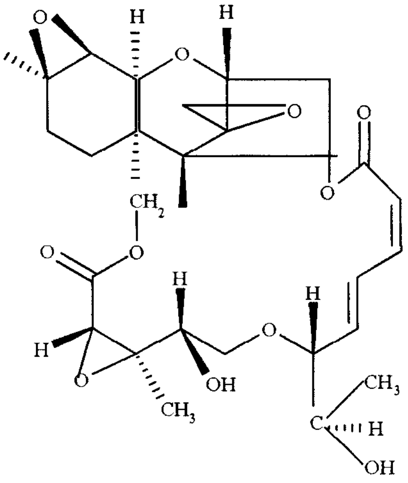


**Fig. (57).** Structure of baccharin

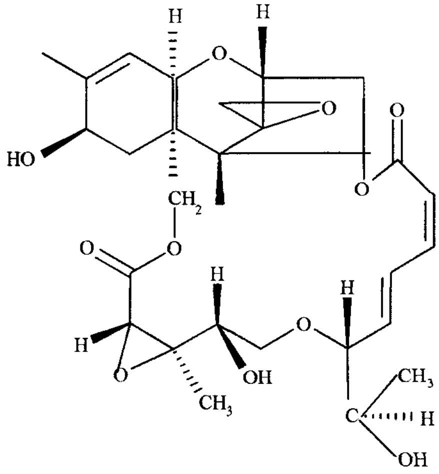


**Fig. (58).** Structure of baccharinol

Bioassay-guided fractionation of an alcoholic extract of *B. megapotamica*, which showed significant activity *in vivo* against P-388 leukemia in mice and *in vitro* against cells derived from human carcinoma of the nasopharynx, yielded four potent antileukemic trichothecenes: baccharin, Fig. **(57)**, baccharinol, Fig. **(58)**, isobaccharinol, Fig. **(59)** and isobaccharin, Fig. **(60)** [84].

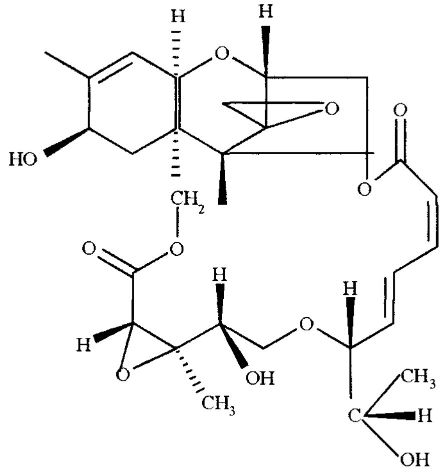


**Fig. (59).** Structure of isobaccharinol

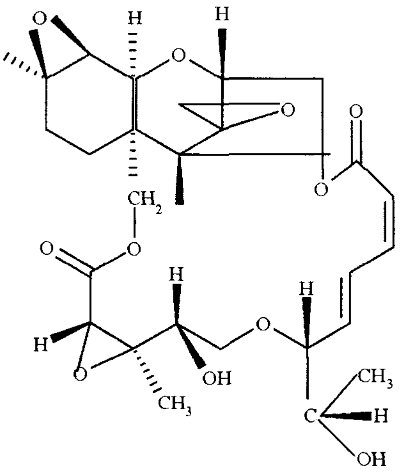


**Fig. (60).** Structure of isobaccharin

It should be noted that while these compounds show potent *in vivo* antileukemic activity against P-388 leukemia in mice, very similar compounds, e.g., roridin D, Fig. **(61)** show no *in vivo* activity. The key difference seems to be the presence of an oxygen substituent in the ring A of the trichothecene nucleus in the baccharinoids. More recently, screening of plants from South America for antineoplastic activity and subsequent assay-guided fractionation resulted in the isolation of several pentacyclic triterpenoids as active constituents from some *Baccharis* spp [85].

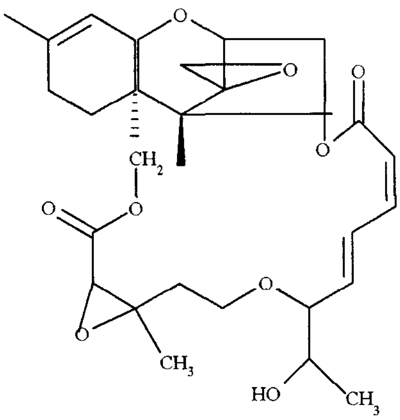


**Fig. (61).** Structure of roridin D

## ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The use of higher plants and their preparations to treat infections is an age-old practice and in times past possibly the only method available. Interest in plants with antimicrobial properties has revived because of the current problems associated with the use of penicillin and other antibiotics. Therapy with several types of antibiotics is frequently accompanied by side effects and microbial resistance. It is currently accepted that the accumulation of secondary metabolites in plants can be a consequence of requirements for chemical defense against microorganisms. Research carried out in the chemical and biological sciences has resulted in much evidence concerning the defensive role of natural products.

Recently, there has been an increased interest in antimicrobial agents from medicinal plants which have been used in folk medicine. A review of the literature concerning the evaluation of plant extracts of the *Baccharis* genus and secondary metabolites isolated from them, has revealed that many studies into their antibacterial and antifungal activities have been carried out in recent years. For example, extracts of 46 plants from 15 families, most of them used in folk medicine, have been screened for antibacterial and antifungal activities against *Bacillus subtilis*, *Micrococcus luteus*,

*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicans, Mucor mucedo* and *Aspergillus niger* [86,87]. Of these, the extracts of *B. crispa* and *B. notosergilla* showed a clear activity against some of the microorganisms tested, including fungi.

Moreover, a microbiological evaluation was conducted on those medicinal plants most frequently used by Tzeltal and Tzotzil communities in the highlands of Chiapas, Mexico [88,89]. The results obtained showed that many of these species, such as *B. serraefolia*, *B. vaccinoides* and *B. genistelloides* induced antimicrobial activity *in vitro* against gram-positive *Staphylococcus aureus*, gram-negative *Escherichia coli* bacteria and *Candida albicans*. Verastegui *et al.* [90] investigated the antimicrobial activity of three widely distributed plants in the vegetation of Northern Mexico and Southern USA, (e.g., *B. glutinosa*) which have been used for several purposes, including treatment of various infectious diseases. The extracts analyzed showed good antimicrobial activity against more than one microorganism. There is also a rich tradition of folk medicine usage in the San Juan province of Argentine. Feresin *et al.* [91] screened 18 extracts from plants used in the traditional medicine of this province for antimicrobial activity against bacteria and fungi. Among them, the hexane and dichloromethane extracts of *B. grisebachii* showed the broadest spectrum of action.

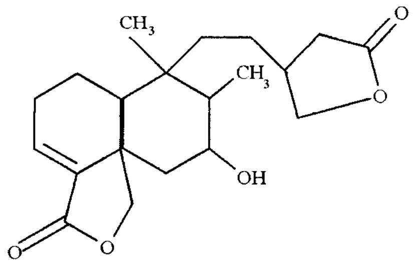


**Fig. (62).** Structure of an antimicrobial diterpene lactone isolated from *Baccharis genistelloides*

A large number of compounds, which are typical constituents of the *Baccharis* genus, were isolated from these species and might be partly or wholly responsible for the antimicrobial effects exhibited by the plants. For example, four compounds, including a flavone, an acetylenic lactone, a

prenylated coumarin and a 3-methyl ether flavone were isolated from *Baccharis pedunculata* (Mill.) Cabr. and were identified as being responsible for antifungal activity against some human pathogenic and phytopathogenic fungi [92]. Dos Santos *et al.* [93] reported the isolation of a diterpene lactone, Fig. **(62)** and a flavone, Fig. **(63)** from *B. genistelloides* which showed antimicrobial activity.

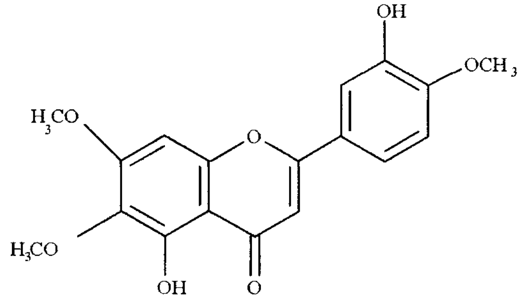


**Fig. (63).** Structure of an antimicrobial flavone isolated from *Baccharis genistelloides*

Other antimicrobial compounds, such as the flavonoids genkwanin, Fig. **(42)** and apigenin, Fig. **(35)** were also reported from *Baccharis* spp [94]. Furthermore, the essential oil of the aerial parts of *B. notosergilla* was tested against eight gram-positive and –negative bacteria and it was found that they exhibit moderate antibacterial activity [95]. Thirty-one constituents were identified, including α-pinene, limonene, Fig. **(33)**, β-caryophyllene, Fig. **(32)** and spathulenol, Fig. **(34)** as the major components. Finally, the triterpenoid oleanolic acid methyl ester isolated from *B. conferta* shows weak antibacterial activity against *Micrococcus luteus* and *Escherichia coli* [96].

## PROTOZOOCIDAL ACTIVITY

The use of protozoocidal compounds from natural vegetation could have a great impact in controllling plants and human diseases of protozoal origin. Chagas' disease is a major health problem in Latin America, which affects about 20 million people in urban or rural areas of the continent. Chagas' disease is caused by *Trypanosoma cruzi*, a protozoon parasite which is mainly transmitted by insect vectors (subfamiliy Triatominae). The growing

urbanization of Chagas' disease has increased the risk of infection in regions that are free of triatomines, where the transmission is exclusively by congenital and blood transfusion mechanisms. The scarcity of serological control for blood donors in endemic areas and the restricted alternative of chemoprofilaxis caused the World Health Organization in 1984 to investigate old and new drugs, and other products which are of potential clinical value.

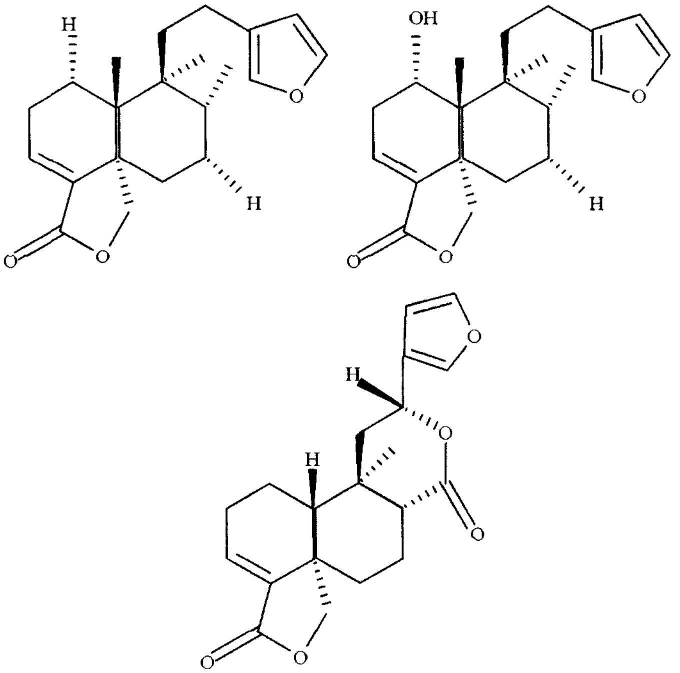

**Fig. (64).** Structure of protozoocidal *neo*-clerodane diterpenoids isolated from *Baccharis* spp.

For Latin American countries comprising Amazonian areas, development of their vast wilderness regions has become a national priority. The family Compositae has been widely used as medicinal plants in Latin American and their antiparasitic properties are well known. Reports on the protozoocidal activity of the genus *Baccharis*, a member of the Compositae, are recorded in the literature. Rojas de Arias *et al.* [97] investigated the trypanocidal effect of crude extracts of some Paraguayan Compositae on the bloodstream forms of

*Trypanosoma cruzi*. A *B. boliviensis* extract showed a total trypomastigote lysis, but better results were found with *B. notosergilla*, which showed a high activity percentage against *Trypanosoma cruzi* trypomastigotes. Many *neo*-clerodane diterpenoids with protozoocidal activity, Fig. **(64)**, which are typical constituents of *Baccharis* spp., have been isolated from plants of this genus [98]. Furthermore, Gonzalez *et al.* [99] have studied the *in vitro* effects of natural products obtained from species of the autochthonous flora of the North of Chile on trypomastigotes of *Trypanosoma cruzi*. A significant trypanocidal effect was observed for three compounds isolated from *B. boliviensis*: two terpenes and a flavone, 4,5-dihydroxy-3,3,6,7,8-pentamethoxyflavone.

Another tropical disease is the mucocutaneous leishmaniasis, which is caused by the protozoan *Leishmania braziliensis*. The settlers and the natives of the endemic regions of cutaneous leishmaniasis recognise this disease represented by a cauker which they attempt to burn with natural or chemical products. In these endemic areas, several investigations have been undertaken on the use of medicinal plants as local treatments for cutaneous leishmaniasis. Fournet *et al.* [100] investigated 40 plants used topically to treat cutaneous leishmaniasis in areas where this disease is endemic, for toxicity *in vitro* against *Leishmania* spp. A number of plants showed an *in vitro* antileishmanial activity, including some species belonging to the genus *Baccharis*, e.g., *B. genistelloides*, *B. dracunculifolia*, *B. incarum*, *B. salicifolia*, *B. tricuneata*., and *Baccharis pentlandii* D. C.

## ANTIVIRAL ACTIVITY

Infectious virus diseases remain an important worldwide problem due to the nature of these infectious agents, which totally depend upon the cell that they infect for their multiplication and survival. This characteristic has made development of effective chemotherapeutic agents for treatment of viral infections very difficult, and as a consequence there are only a few antiviral drugs available for the cure of virus diseases. During the last years, the search for natural compounds with antiviral activity has been intensified. Antiviral screening programmes of indigenous medicinal plants are very important, not

only for the contribution they make to the knowledge of autochthonous flora, but also as a way of solving the problems related to the treatment of virus diseases [101].

A few reports have been recorded in the literature on the antiviral activity of several members from *Baccharis* genus. Waschsman *et al.* [102] initiated a screening programme for antiviral activity in higher plants used in Argentinian folk medicine belonging to different botanical families, including Compositae. It was found that the replication of several viruses, such as vesicular stomatitis virus (VSV) and poliovirus was significantly inhibited by *B. crispa* and *B. notosergilla*. Also, certain medicinal plants from the South Cordoba province of Argentina were assayed for antiviral activity using Vero cells-pseudorabies virus strain RC/79 (herpes suis virus) system [103]. Some plant extracts, including ethanolic extract from *B. articulata*, were able to inhibit the viral infectivity. In the search for new sources of antiviral substances, ethanolic and aqueous extracts of several plant species used in the traditional medicine of Bolivia and Ecuador have been tested for their antiviral activity against herpes simplex virus type I (HSV-1), VSV and poliovirus type 1 [104,105]. One of the Bolivian plants, namely *B. genistelloides*, was active against two different viruses, HSV-1 and VSV, while the Ecuatorian *B. trinervis* inhibited HSV-1 replication by 100% without showing cytotoxic effects. Additionally, this plant, *B. trinervis*, exhibited antiviral activity against human immunodeficiency virus in MT-2 infected cells [106].

In most cases, the chemical nature of the active principles from these extracts has not been well established, but for some plants it has been demonstrated that the reported antiviral activity is associated with phenolics, flavonoids, tannins, carbohydrates or alkaloids, among others. However, a few reports of the chemical structure of antiviral compounds isolated from *Baccharis* plants are recorded in the literature. Macrocyclic trichothecenes produced by *B. coridifolia*, such as roridins, Fig. **(61)**, verrucarins, satratoxins and baccharinoids, Fig. **(57-60)** have been reported to possess antiviral activity against herpes simplex virus type II (HSV-2) and the arenavirus Junin, the ethiological agent of Argentine hemorrhagic fever [107,108].

## ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES

The nonsteroidal anti-inflammatory drugs (NSAIDs) currently in use are characterized by their ability to relieve pain, fever and inflammation, but also by their well-known side effects. In the past few years, anti-inflammatory compounds having a profile of action on the arachidonic acid cascade metabolism different from that of the classical NSAIDs have been reported. A greatly increased understanding of the biochemistry of pathophysiological inflammatory reactions, together with the establishment of many new *in vitro* test systems for screening, have stimulated the search for new antiphlogistic agents. Furthermore, the participation of reactive oxygen species (ROS) in the mechanisms of many chronic and acute inflammatory diseases has focussed the attention of researchers on the potential protective functions of antioxidants.

In the search for new anti-inflammatory compounds, special interest has been directed to herbal based products reputed in traditional medicine to have beneficial effects in inflammatory disorders. Various investigation groups initiated anti-inflammatory screening programmes on plants used in traditional medicine from all over the world in the treatment of several pathologies as anti-inflammatory agents, although other uses have also been suggested. Some of these studies concern species belonging to the genus *Baccharis*. For example, *B. articulata*, *B. crispa* and *B. genistelloides* are used in the form of decoctions in the traditional medicine of South America for the treatment of rheumatism, liver diseases, wounds and ulcers. Gene *et al.* [109] investigated the effect of the aqueous extracts of these plants by using the carrageenan foot edema test. Aqueous extracts administered *i.p.* produced a marked inhibition of edema, but none of the three extracts was active when administered by the oral route. Similarly, the anti-inflammatory activities of organic extracts from the aerial parts of *B. medullosa* and *B. rufescens*, species growing in the western semi-arid area of Argentina, were investigated in mice subjected to carrageenan induced paw edema [110]. These results indicate that the most effective extracts were the hexane from *B. medullosa*, and the acetone and chloroform extracts from *B. rufescens*. Other species from this genus, such as *B. incarum* and *B. tucumanensis*

showed a marked anti-inflammatory action in the carrageenan foot edema test, and on the croton oil induced mouse ear edema test [111,112].

Furthermore, reports on the antioxidant activity of *Baccharis* genus are recorded in the literature. Desmarchelier *et al.* [113] screened the aqueous extracts of 19 medicinally reputed Argentine plants *in vitro* for their antioxidant or prooxidant activities utilizing *tert*-butyl hydroperoxide-initiated chemiluminescence and thiobarbituric acid assays. From plants belonging to the genus *Baccharis*, *B. coridifolia* and *B. crispa* showed the highest antioxidant activity in both assay systems. Ethanolic extracts from 15 plant species used in traditional medicine in Ecuador were also evaluated for antioxidant activity [114]. The extracts were tested *in vitro* for their ability to inhibit lipid peroxidation and to scavenge superoxide and hydroxy radicals, and it was found that, among others, extracts of *B. trinervis* were active as antioxidant. More recently, Perez *et al.* [115] examined the activity of some medicinal plant extracts on oxidative stress in human blood leukocytes stimulated by several ROS-producing agents. Among them, the best inhibitory activity was shown by the dichloromethane extracts of *B. grisebachii* that was active in all the assays.

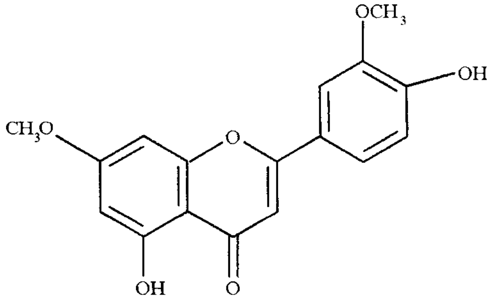


**Fig. (65).** Structure of luteolin-7-3'-dimethylether

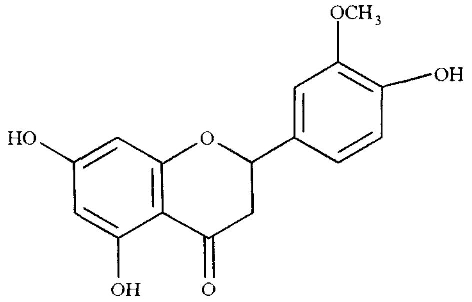


**Fig. (66).** Structure of homoeriodictyol

Different chemical constituents, including flavonoids, have been considered as the active principles of many anti-inflammatory plants. From *Baccharis calliprinos* Grised and *Baccharis rhetinoides* Meyer & Walp, growing in the Cuyo region of Argentina, Gianello *et al.* [116] reported the isolation and identification of several anti-inflammatory flavonoids, such as luteolin-7,3'-dimethylether, Fig. **(65)**, homoeriodictyol, Fig. **(66)** and eriodictyol-3',4'-dimethylether, Fig. **(67)**.

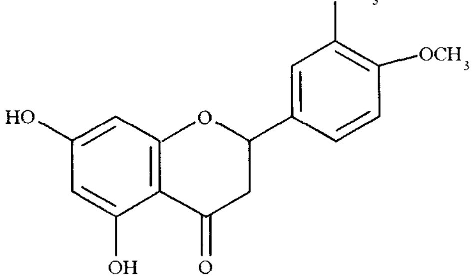


**Fig. (67).** Structure of eriodictyol-3',4'-dimethylether

Other flavonoids, e.g. rutin, Fig. **(68)** have been isolated as anti-inflammatory constituents from *B. genistelloides* [117].

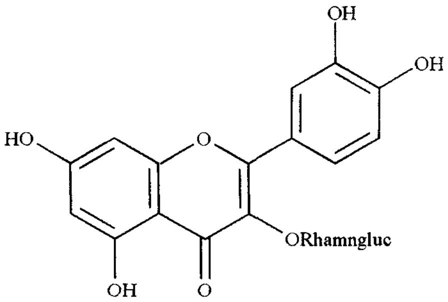


**Fig. (68).** Structure of rutin

However, a saponin mixture, an unusual constituent of *Baccharis* genus, in which echinocystic acid, Fig. **(69)** is the major aglycone, has been found to be the main active principle of this plant.

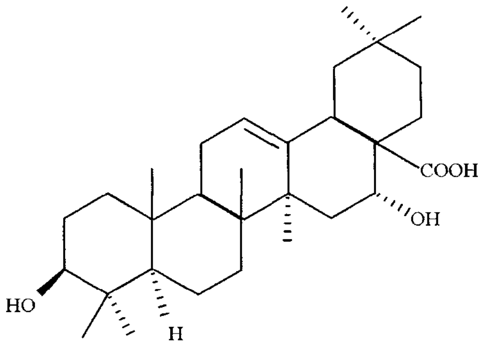


**Fig. (69).** Structure of echinocystic acid

## OTHER BIOLOGICAL ACTIVITIES

Several other pharmacological actions have been documented from the *Baccharis* genus, some of which may be related to their traditional use. In developing countries, acute diarrhoeal disease is the major cause of mortality and morbidity in infants. In an interdisciplinary approach with the emphasis placed on ethnomedical and ethnobotanical studies, 141 plant species used for the treatment of gastrointestinal diseases, especially diarrhoea associated

with gastrointestinal cramps, and respiratory diseases in the highlands of Chiapas, Mexico, were experimentally evaluated [118,119]. This was done in order to determine their spasmolytic activity on guinea pig ileum. The genus *Baccharis* was found to be the most interesting genus screened. All the tested *Baccharis* (*B. glutinosa*, *B. serraefolia*, *B. trinervis*, *B. vaccinoides* and *B. heterophylla*) leaf extracts were active, regardless of the locality where the samples were collected, while the stem extracts were all inactive.

Tortoriello and Aguilar-Santamaria [120] investigated in detail the spasmolytic mechanisms of action of one of these plants, *B. serraefolia*. The results suggest that the extract does not interact with classical receptors and its effects, at least that produced on smooth muscle, may be due to a probable interference with calcium influx and/or calcium release from an intracellular store. Different chemical compounds, such as clerodane diterpenes and flavonoids, which are typical constituents from the *Baccharis* genus, have been considered as the active principles of many spasmolytic drugs. A bioassay-monitored fractionation of an extract from *B. genistelloides* yielded a mixture that blocked the calcium-induced contractions of KCl-depolarized rat portal vein preparations [121]. Pharmacological tests of two pure compounds isolated from the mixture revealed a dilactonic clerodane-diterpene, Fig. **(70)** as the active compound.

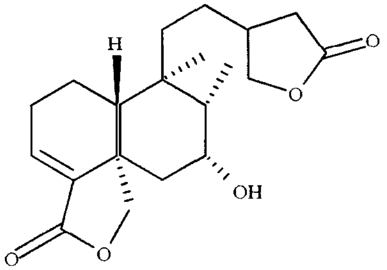


**Fig. (70).** Structure of an spasmolytic clerodane-diterpene isolated from *Baccharis genistelloides*

From *B. conferta*, an endemic species of the Nahua of the Mexican state of Veracruz where it is used in the treatment of a variety of gastrointestinal illnesses, several flavonoids, such as pectolinaringenin, Fig. **(36)** and cirsimaritin, Fig. **(40)** were isolated [96]. These flavonoids play an important role in the antispasmodic activity of this indigenous drug.

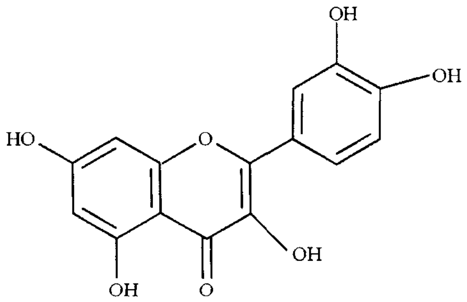


**Fig. (71).** Structure of quercetin

Other pharmacological activities reported from the *Baccharis* genus are presented below. *Baccharis triptera* Mart., a small plant widely distributed in Brazil, relieves gastrointestinal disorders by reducing acid secretion and gastrointestinal hiperactivity [122]. Preparations from *B. genistelloides* are used in South American folk medicine, among others, in cases of liver diseases. Soicke and Leng-Peschlow [123] isolated and characterized the flavonoids of this plant proceeding from an antihepatotoxic crude extract: quercetin, Fig. **(71)**, luteolin, nepetin, Fig. **(72)**, apigenin, Fig. **(35)** and hispidulin, Fig. **(73)**. The efficacy of the crude extract seems to be explained to a large extent by the identified compounds.

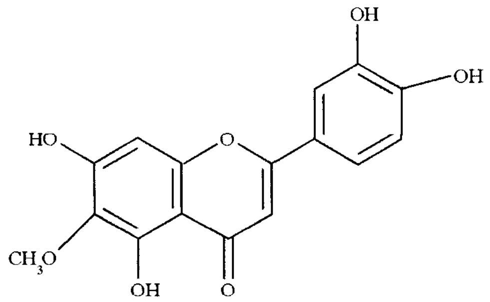


**Fig. (72).** Structure of nepetin

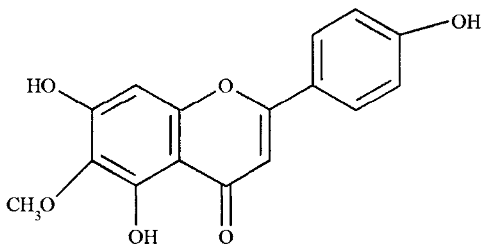


**Fig. (73).** Structure of hispidulin

## TOXICOLOGICAL CONSIDERATIONS

Medicinal plants, plants used for eating purposes and other natural products, are mainly complex products with several components with different chemical and pharmacological characteristics. In addition, many of these products are also sold as dietary supplements, but scientific information about their safe and effective use is hard to find because limited toxicological data are available on herbal remedies and the support of rigorous clinical studies is lacking. A few reports of the toxicological evaluation in *Baccharis* species are available.

*B. genistelloides* is widely used in folk medicine in the form of infusion for its anti-inflammatory, healing and digestive properties. Pedrazzi *et al.* [124] investigated the effects on bone marrow, pancreas, liver, bile duct, cardiac function and serum lipid after oral administration of this drug. Under the experimental conditions employed, *B. genistelloides* infusion caused no changes that might suggest a toxic side effect. Similar results have been obtained with other species from *Baccharis* genus, such as *Baccharis cylindrica* (Less.) D. C., *Baccharis microcephala* (Less.) D. C., *Baccharis usterii* Heering and *Baccharis ochracea* Spreng. [125,126]. Furthermore, aqueous extracts of several species used in Brazilian popular medicine were screened for the presence of mutagenic activity in the Ames test (*Salmonella*/microsome) [127]. Among them, positive results were obtained for *Baccharis anomala* D. C. with microsomal activation. Several *Baccharis* species used as the raw material for the preparation of phytotherapeutic drugs, e.g., *B. genistelloides*, *B. crispa*, *B. articulata* and *Baccharis*

*myriocephala* D. C., were analysed by botanical, chemical, physico-chemical and technological methods to ensure their adequate quality control method [128-130].

Toxic disease in livestock (equine, ovine and bovine) caused by the shrubs *B. coridifolia* and *Baccharis artemisioides* D. C. is very common in Argentina, particularly during the flowering season [131,132]. The direct and indirect economic loss of farm animals associated with these toxic plants is roughly estimated at 50 million dollars a years [133]. Previously, another Brazilian shrub, *B. megapotamica*, was reported to contain a series of potent cytotoxic agents belonging to the trichothecene complex of antibiotics [53]. This was surprising because the trichothecene mycotoxins heretofore had been found to be produced only by soil fungi. The trichothecenes are an important group of fungal toxins whose role in mycotoxicoses and plant pathogenicity is currently of interest. Furthermore, the trichothecenes exhibit exceptionally high toxicity toward eucaryotic organisms, including high phytotoxicity.

Unlike all other plants tested to date, two Brazilian plants (*B. megapotamica* and *B. coridifolia*) are virtually immune (both in cell culture and in whole plant) to the toxic effects of these potents phytotoxins. Recent data suggest that these plants produce these toxins de novo and, in the case of *B. coridifolia*, the female plant following pollination often contains >10 times the amount found in the male plant. The principal metabolites, roridins, Fig. **(61)**, in the female *B. coridifolia* are identical to the major metabolites produced by the fungi *Myrothecium verrucaria* and *Myrothecium roridum*. Hybrids resulting from crosses between several *Baccharis* species are being tested for their tolerance to trichothecenes as well as their ability to metabolize the toxins [134].

Several studies investigated the *in vivo* toxicity of macrocyclic trichothecenes [135]. It is assumed that their toxic effects are based on the inhibition of protein synthesis, with a slowly progressing respiratory depression and paralysis of skeletal muscles. The epidermal remains of these plant species that produce acute intoxication in rumiants were also quantified by microhistological analysis in the gastrointestinal content of sheep which had been experimentally poisoned [136].

## OTHER USES

Apart from the pharmacological activities reported from species of the *Baccharis* genus, some of these may be useful as an instrument for the restoration of tropical mountain rainforest. In the neotropics, extensive areas of mild-altitude tropical mountain forest have been deforested for the establishment of agricultural lands, particularly coffee plantations. Over the last 150 years, a large proportion of forest in Latin America has been converted to pastures. When these pastures are abandoned, grasses may slow re-establishment of woody species and limit forest regeneration. Once pastures are abandoned, the rate of forest recovery depends on the degree of site degradation. Forest regeneration in areas subject to intense grazing, long-term use and soil erosion can be very slow or may never occur. To accelerate forest regeneration in these productive grasslands, competition from grasses needs to be reduced.

In these ecosystems, most wind-dispersed species are weedy shrubs or small trees in the Compositae that normally occur in frequently disturbed areas and are not the pioneer species in mature forest gaps. The establishment of these wind-dispersed species can enhance forest regeneration by shading the grass and creating a microclimate for the germination and establishment of forest species. Vegetation surveys have been carried out with species belonging to the genus *Baccharis* endemic to the Argentine Pampas [137], Colombia [138,139], the Bolivian Altiplano [140] and to Southeastern Arizona [141].

## ABBREVIATIONS

VSV = Vesicular Stomatitis Virus
HSV-1 = Herpes Simplex Virus Type I
HSV-2 = Herpes Simplex Virus Type II
NSAIDS = Nonsteroidal Anti-Inflammatory Drugs
ROS = Reactive Oxygen Species

## ACKNOWLEDGEMENTS

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## REFERENCES


[1] Heinrich. M.; *Phytother. Res,* **2000,** *14,* 479-488.

[2] Nesom, G.L. *Generic Conspectus of the Tribe Astereae (Asteraceae) in North America and Central America, the Antilles and Hawaii,* Botanical Research Institute of Texas, Chapel Hill: USA, **2000.**

[3] Heinrich, M.; Robles, M.; West, J.E.; Ortiz de Montellano, B.R.; Rodriguez, E.; *Annu. Rev. Pharmacol. Toxicol,* **1998,** *38,* 539-565.

[4] Garcia-Alvarado, J.S.; Verde-Star, M.J.; Heredia, N.L.; *J. Herbs, Spices Med. Plants,* **2001,** *8,* 37-89.

[5] Arqueta-Villamar, A.; Cano-Asseleih, L.M.; Rodarte, M.E. *Atlas de las Plantas de la Medicina Tradicional Mexicana,* Instituto Nacional Indigenista: Mexico D.F. **1994.**

[6] Del Amo, R.S. *Plantas Medicinales del Estado del Veracruz Xalapa, Veracruz,* Instituto Nacional de Investigaciones sobre Recursos Bioticos: Veracruz, **1979.**

[7] Weimann, C.; Heinrich. M.; *Bot. Acta,* **1997,** *11,* 62-72.

[8] Lucca, M.; Zalles, J. *Flora Medicinal Boliviana: Diccionario Enciclopédico,* Ed. Los Amigos del Libro: La Paz, **1992.**

[9] Berlin, B.; Berlin, E.A.; Breedlove, D.E.; Duncan, T.; Jara, V.M.; Laughlic, R.M.; Velasco, T. *La Herbolaria Medica. Tzeltal-Tzotzil en las Altas de Chiapas,* Procomith Gobierno del Estado de Chiapas: Mexico, **1990.**

[10] Alcorn, J.B. *Huastec Mayan Ethnobotany,* Univ. Texas Press: Austin, **1984**.

[11] Berlin, E.A.; Berlin, B. *Medical Ethnobotany of the Highland Maya of Chiapas, Mexico,* Princeton Univ. Press: Princeton, **1996.**

[12] Zardini, E.M.; *Acta Farm. Bonaerense,* **1984,** *3,* 77-99.

[13] Gupta, M.P. *270 Plantas Medicinales Iberoamericanas,* Convenio Andrés Bello: Colombia, **1995.**

[14] Jatem-Lásser, A.; Ricardi, M.S.; Adamo, G.; *Phytother. Res,* **1998,** *12,* 553-559.

[15] Diaz, S. ; *Rev. Acad. Colomb. Ciens,* **1988,** *16,* 23.

[16] Simoes, C.M.O.; Mentz, L.A.; Schenkel, E.P.; Irgang, B.E.; Stehmann, J.R. *Plantas da Medicina Popular no Rio Grande do Sul,* Ed. da Universidade: Porto Alegre, **1986.**

[17] Sousa, M.P.; Matos, N.E.O.; Matos, M.F.J.A.; Machado, M.I.L.; Craveiro, A.A. *Constituintes Quimicos Activos de Plantas Medicinais Brasileiras,* Fortaleza Ediçoes UFC: Brasil, **1991.**

[18] Killeen, T.J.; Garcia, E.; Beck, S.G. *Guia de Arboles de Bolivia,* Quipus S.R.L.: La Paz, **1993.**
[19] Kuroyanagi, M.; Uchida, K.; Ueno, A.; Satake, M.; Shimamura, K.; *Phytochemistry,* **1993,** *34,* 1377-1384.
[20] Zdero, C.; Bohlmann, F.; King, R.M.; Robinson, H.; *Phytochemistry,* **1986,** *25,* 2841-2855.
[21] Warning, V.; Bohlmann, F.; Sanchez, V.H.; Del Rio, S.E.; Dominguez, X.A.; *Rev. Latinoam. Quim,* **1986,** *17,* 199-20.
[22] Stapel, G.; Menssen, H.G.; Snatzke, G.; Forschungslab, A.; Natlermann, M.; *Planta Med,* **1980,** *39,* 366-374.
[23] Gianello, J.C.; Giordano, O.S.; *Rev. Latinoam. Quim,* **1982,** *13,* 76-78.
[24] Wagner, H.; Seitz, R.; Chari, V.M.; Lotter, H.; Herz, W.; *Tetrahedron Lett,* **1977,** *35,* 3039-3040.
[25] Bohlmann, F.; Zdero, C.; Grenz, M.; Dhar, A.K.; Robinson, H.; King, R.M.; *Phytochemistry,* **1981,** *20,* 281-286.
[26] Li, C.J.; Ahmed, A.A.; Arias, A.D.C.; Mabry, T.J.; *Phytochemistry,* **1997,** *45,* 571-574.
[27] Zdero, C.; Bohlmann, F.; Niemeyer, H.W.; *Phytochemistry,* **1991,** *30,* 1597-1601.
[28] San Martin, A.; Givochich, A.; Castillo, M.; *Phytochemistry,* **1986,** *25,* 264-266.
[29] Gambarro, V.; Chamy, M.C.; Garbarino, J.A.; San Martin, A.; Castillo, M.; *Phytochemistry,* **1986,** *25,* 2175-2177.
[30] Rivera, A.P.; Arancibia, L.; Castillo, M.; *J. Nat. Prod,* **1989,** *52,* 433-435.
[31] Arriaga-Giner, F.J.; Wollenweber, E.; Schober, I.; Dostal, P.; Braun, S.; *Phytochemistry,* **1986,** *25,* 719-721.
[32] Morales, G.; Mancilla, A.; Gallardo, O.; Trujillo, R.; Loyola, L.; Breton, J.L.; *Bol. Soc. Chil. Quim,* **1990,** *35,* 257-263.
[33] Zdero, C.; Bohlmann, F.; Solomon, J.C.; King, R.M.; Robinson, H.; *Phytochemistry,* **1989,** *28,* 531-542.
[34] Jakupovic, J.; Schuster, A.; Ganzer, U.; Bohlmann, F.; Boldt, P.E.; *Phytochemistry,* **1990,** *29,* 2217-2222.
[35] Herz, W.; Pilotti, A.M.; Söderholm, A.C.; Shuhama, I.K.; Vichnewski, W.; *J. Org. Chem,* **1977,** *42,* 3913-3917.
[36] Kuroyanagi, M.; Fujita, K.; Kazaoka, M. ; Matsumoto, S.; Veno, A.; Fukushima, S.; Katsuoka, M.; *Chem. Pharm. Bull,* **1985,** *33,* 5075-5078.
[37] Suttisri, R.; Kinghorn, A.D.; Wright, A.D.; Sticcher, O.; *Phytochemistry,* **1994,** *35,* 443-446.
[38] Bohlmann, F.; Banerjee, S.; Jakupovic, J.; Grenz, M.; Misra, L.N.; Schmeda-Hirschmann, G.; King, R.M.; Robinson, H.; *Phytochemistry,* **1985,** *3,* 511-515.
[39] Bohlmann, F.; Kramp, W.; Grenz, M.; Robinson, H.; King, R.M.; *Phytochemistry,* **1981,** *20,* 1907-1913.

[40] Zdero, C.; Bohlmann, F.; Niemeyer, H.W.; *Phytochemistry,* **1990,** *29,* 2611-2616.
[41] Wachter, G.A.; Montenegro, G.; Timmermann, B.N.; *J. Nat. Prod,* **1999,** *62,* 307-308.
[42] Cifuente, D.A. ; Tonn, C.E. ; Giordano, O.S. ; *Nat. Prod. Lett,* **2001,** *15,* 425-431.
[43] Faini, C.L.F.; Castillo, M.; *Phytochemistry,* **1990,** *29,* 324-325.
[44] Bohlmann, F.; Scheidges, C.; Zdero, C.; King, R.M.; Robinson, H.; *Phytochemistry,* **1984,** *23,* 1109-1111.
[45] Bohlmann, F.; Kramp, W.; Jakupovic, J.; Robinson, H.; King, R.M.; *Phytochemistry,* **1982,** *21,* 399-403.
[46] San Martin, A.; Rovirosa, J.; Castillo, M.; *Phytochemistry,* **1983,** *22,* 1461-1463.
[47] Weyerstahl, P.; Marschall-Weyerstahl, H.; Christiansen, C.; *Planta Med,* **1990,** *56,* 542.
[48] Norte, M.; Cataldo, F.; Sanchez, A.; Gonzalez, A.G.; Rivera, P.; Castillo, M.; *Tetrahedron Lett,* **1993,** *34,* 5143-5146.
[49] Rivera, A.P.; Castillo, M.; Rodriguez, M.L.; *Acta Crystallogr,* **1988,** *44,* 161-163.
[50] Parodi, F.J.; Fischer, N.H.; *Phytochemistry,* **1988,** *27,* 2987-2988.
[51] Jarvis, B.B.; Pena, N.B.; Cömezoglu, S.N.; Rao, M.M.; *Phytochemistry,* **1986,** *25,* 533-535.
[52] Jarvis, B.B.; Comeziglu, S.N.; Ammon, H.L.; Breedlove, C.K.; Miller, R.W.; Woode, M.K.; Streelmon, D.R.; Sneden, A.T.; Dailey, R.G.Jr.; Kupchan, S.M.; *J. Nat. Prod,* **1987,** *50,* 815-828.
[53] Jarvis, B.B.; Midiwo, J.O.; Bean, G.A.; Bassam Aboul-Nasr, M.; Barros, C.S.; *J. Nat. Prod,* **1988,** *51,* 736-744.
[54] Anthonsen, T.; Bruun, T.; Hemmer, E.; Holme, D.; Lamvik, A.; Sunde, E.; Sorensen, N.A.; *Acta Chem. Scand,* **1970,** *24,* 2479-2488.
[55] Dominguez, X.A.; Sanchez, H. ; Merijanian, B.A. ; Rojas, M.P. ; *Phytochemistry,* **1972,** *11,* 2628.
[56] Mo, F.; Anthonsen, T.; Bruun, T.; *Acta Chem. Scand,* **1972,** *26,* 1287-1288.
[57] Nurnberg, V.; Fujiwara, F.Y.; Marsaioli, A.J.; *Magn. Reson. Chem,* **1998,** *36,* 766-768.
[58] Labbe, C.; Rovirosa, J.; Faini, F.; Mahu, M.; San Martin, A. ; Castillo, M. ; *J. Nat. Prod,* **1986,** *49,* 517-518.
[59] Rivera, A.P.; Calle, J.; Luis, J.G.; Joseph-Nathan, P.; *Fitoterapia,* **1994,** *65,* 285.
[60] Arisawa, M.; Kinghorn, A.D.; Cordell, G.A.; Phoebe, C.H.; Fansworth, N. R.; *Planta Med,* **1985,** *6,* 544-545.
[61] Daily, A.; Wagner, H.; Seligmann, O.; *Fitoterapia,* **1984,** *55,* 236-238.
[62] El Dahmy, S.I.; *Sci. Pharm,* **1994,** *62,* 67-70.
[63] Weyerstahl, P.; Christiansen, C.; Marschall, H.; *Flavour Fragance J,* **1996,** *11,* 15-23.

[64] Loayza, I.; Collin, G.; Gagnon, M.; Dellacassa, E.; *Riv. Ital. EPPOS,* **1993,** *4,* 728-736.

[65] Zunino, M.P.; Novillo-Newton, M.; Maestri, D.M.; Zygadlo, J.A.; *Flavour Fragance J,* **1997,** *12,* 405-407.

[66] Zunino, M.P.; Lopez, M.L.; Faillaci, S.M.; Lopez, A.G.; Ariza-Espinar, L.; Zydgadlo, J.A.; *Flavour Fragance J,* **2000,** *15,* 151-152.

[67] Zunino, M.P.; Novillo-Newton, M.; Maestri, D.M.; Zygadlo, J.A.; *Planta Med,* **1998,** *64,* 86-87.

[68] Van Baren, C.M.; Lira, P.D.; Bandoni, A.L.; Fortunato, R.; Mizrahi, I.; Juarez, M.; *J. Essential Oil Res,* **2002,** *14,* 183-186.

[69] Frizzo, C.D.; Serafini, L.A.; Dellacassa, E.; Lorenzo, D.; Mogna, P.; *Flavour Fragance J,* **2001,** *16,* 286-288.

[70] Arriaga, F.J.; Borges, J.; Manresa, M.T.; Peña, S.; Rodriguez, F.; *Rev. Latinoam. Quim,* **1982,** *13,* 47-49.

[71] Sharp, H.; Bartholomew, B.; Bright, C.; Latif, Z.; Sarker, S.D.; Nash, R.J.; *Biochem. Syst. Ecol,* **2001,** *29,* 105-107.

[72] Gianello, J.C.; Giordano, O.S.; *Ann. Asoc. Quim. Argent,* **1987,** *75,* 1-3.

[73] Rendon, W.; Vila, J.L.; *Rev. Boliv. Quim,* **1995,** *12,* 13-15.

[74] Kupchan, S.M.; Baverschmidt, E.; *Phytochemistry,* **1971,** *10,* 664-666.

[75] Nagatani, Y.; Warashina, T.; Noro, T.; *Chem. Pharm. Bull,* **2001,** *49,* 1388-1394.

[76] Nagatani, Y.; Warashina, T.; Noro, T.; *Chem. Pharm. Bull,* **2002,** *50,* 583-589.

[77] Cordell, G.A.; Beecher, C.W.; Pezzutto, J.M.; *J. Ethnopharmacol,* **1991,** *32,* 117-133.

[78] Mongelli, E.; Martino, V.; Coussio, J.; Ciccio, G.; *Int. J. Pharmacog,* **1996,** *34,* 249-254.

[79] Mongelli, E.; Desmarchelier, C.; Rodriguez Talou, J.; Coussio, J.; Ciccio, G.; *J. Ethnopharmacol,* **1997,** *58,* 157-163.

[80] Mongelli, E.; Pampuro, S.; Coussio, J.; Salomon, H.; Ciccio, G.; *J. Ethnopharmacol,* **2000,** *71,* 145-151.

[81] Nakasugi, T.; Komai, K.; *J. Agric. Food Chem,* **1998,** *46,* 2560-2564.

[82] Fullas, F.; Hussain, R.A.; Bordas, E.; Pezzutto, J.M.; Soejarto, D.D.; Kinghorn, A.D.; *Tetrahedron,* **1991,** *47,* 8515-8522.

[83] Fullas, F.; Hussain, R.A.; Chai, H.B. ; Pezzutto, J.M.; Soejarto, D.D.; Kinghorn, A.D.; *J. Nat. Prod,* **1994,** *57,* 801-807.

[84] Kupchan, S.M.; Streelmen, D.R.; Jarvis, B.B.; Dailey, R.G.Jr.; Sneden, A.T.; *J. Org. Chem,* **1977,** *42,* 4221-4225.

[85] Wachter, G.A.; Valcic, S.; Flagg, M.L.; Franzblau, S.G.; Montenegro, G.; Suarez, E.; Timmermann, B.N.; *Phytomedicine,* **1999,** *6,* 341-345.

[86] Gutkind, G.O.; Martino, V.; Graña, N.; Coussio, J.D.; De Torres, R.A.; *Fitoterapia,* **1981,** *52,* 213-218.

[87] Palacios, P.; Gutkind, G.; Rondina, R.V.; De Torres, R.; Coussio, J.D.; *Planta Med,* **1983,** *49,* 128.
[88] Meckes, M.; Villareal, M.L.; Tortoriello, J.; Berlin, B.; Berlin, E.A.; *Phytother. Res,* **1995,** *9,* 244-250.
[89] Melo, S.F.; Soares, S.F.; Da Costa, R.F.; Da Silva, C.R.; De Oliveira, M.B.N.; Bezera, R.J.A.C.; Caldeira de Araujo, A.; Bernardo-Filho, M.; *Mut. Res,* **2001,** *496,* 33-38.
[90] Verastegui, M.A.; Sanchez, C.A.; Heredia, N.L.; Garcia-Alvarado, J.S.; *J. Ethnopharmacol,* **1996,** *52,* 175-177.
[91] Feresin, G.E.; Tapia, A.; Lopez, S.N.; Zacchino, S.A.; *J. Ethnopharmacol,* **2001,** *78,* 103-107.
[92] Rahalison, L.; Benathan, M.; Monod, M.; Frenk, E.; Gupta, M.P.; Solis, P.N.; Fuzzati, N.; Hostettmann, K.; *Planta Med,* **1995,** *61,* 360-362.
[93] Dos Santos, D.; Sarli, S.J.; Vichnewski, W.; Bulhoes, M.S.; De Freitas, H.; *Rev. Fac. Farm. Odontol. Ribeirao Preto,* **1980,** *17,* 43-47.
[94] Recio, M.C.; Rios, J.L.; Villar, A.; *Phytother. Res,* **1989,** *3,* 117-125.
[95] Cobos, M.I.; Rodriguez, J.L.; Oliva, M.M.; Demo, M.; Faillaci, S.M.; Zygadlo, J.A.; *Planta Med,* **2001,** *67,* 84-86.
[96] Weimann, C.; Goransson, U.; Pongprayoon-Claeson, U.; Claeson, P.; Bohlin, L.; Rimpler, H.; Heinrich. M.; *J. Pharm. Pharmacol,* **2002,** *54,* 99-104.
[97] Rojas de Arias, A.; Ferro, E.; Inchausti, A.; Ascuru, M.; Acosta, N.; Rodriguez, E.; Fournet, A.; *J. Ethnopharmacol,* **1995,** *45,* 35-41.
[98] Sosa, M.E.; Tonn, C.E.; Giordano, O.S.; *J. Nat. Prod,* **1994,** *57,* 1262-1265.
[99] Gonzalez, J.; Sagna, H.; Araya, J.; Loyola, A.; Morales, G.; Pereira, J.; Estrada, M.; *Phytother. Res,* **1990,** *4,* 1-4.
[100] Fournet, A.; Barrios, A.A.; Muñoz, V.; *J. Ethnopharmacol,* **1994,** *41,* 19-37.
[101] Vlietinck, A.J.; Vanden Berghe, D.A.; *J. Ethnopharmacol,* **1991,** *32,* 141-154.
[102] Wachsman, M.B.; Coto, E.; Martino, V.; *Fitoterapia,* **1988,** *59,* 422-424.
[103] Zanon, S.M.; Ceriatti, F.S.; Rovera, M.; Sabini, L.J.; Ramos, B.A.; *Rev. Latinoam. Microbiol,* **1999,** *41,* 59-62.
[104] Abad, M.J.; Bermejo, P.; Gonzales, E.; Iglesias, I.; Irurzun, A.; Carrasco, L.; *Gen. Pharmac,* **1999,** *32,* 499-503.
[105] Abad, M.J.; Bermejo, P.; Sanchez Palomino, S.; Chiriboga, X.; Carrasco, L.; *Phytother. Res,* **1999,** *13,* 142-146.
[106] Sanchez Palomino, S.; Abad, M.J. ; Bedoya, L.M. ; Garcia, J. ; Gonzales, E. ; Chiriboga, X.; Bermejo, P.; Alcami, J.; *Biol. Pharm. Bull,* **2002,** *25,* 1147-1150.
[107] Okazaki, K.; Yoshizawa, T.; Kimura, S.; *Agric. Biol. Chem,* **1989,** *53,* 1441-1443.
[108] Garcia, C.C.; Rosso, M.L.; Bertoni, M.D.; Maier, M.S.; Damonte, E.B.; *Planta Med,* **2002,** *68,* 209-212.
[109] Gene, R.M.; Marin, E.; Adzet, T.; *Planta Med,* **1992,** *58,* 565-566.

[110] Cifuente, D.A.; Simirgiotis, M.J.; Favier, S.; Rotelli, A.E.; Pelzer, L.E.; *Phytother. Res*, **2001,** *15*, 529-531.
[111] Perez, F.; Marin, E.; Adzet, T.; *Phytother. Res*, **1995,** *9*, 145-146.
[112] Muschietti, L.; Martino, V.; Ferrara, G.; Coussio, J.; Segura, L.; Cartana, C.; Cañigueral, S.; Adzet, T.; *Phytother. Res*, **1996,** *10*, 84-86.
[113] Desmarchelier, C.; Novoa, M.J.; Coussio, J.; Ciccia, G. ; Boveris, A. ; *Int. J. Pharmacog*, **1997,** *35*, 116-120.
[114] De las Heras, B.; Slowing, K.; Benedi, J.; Carretero, E.; Ortega, T.; Toledo, C.; Bermejo, P.; Iglesias, I.; Abad, M.J.; Gomez-Serranillos, P.; Liso, P.A.; Villar, A.; Chiriboga, X.; *J. Ethnopharmacol*, **1998,** *61*, 161-166.
[115] Perez, F.; Marin, E.; Adzet, T.; Cañigueral, S.; *Phytomedicine*, **2001,** *8*, 31-38.
[116] Gianello, J.C.; Cifuente, D.A.; Giordano, O.S.; Tonn, C.E.; *Acta Farm. Bonaerense*, **1999,** *18*, 99-102.
[117] Gene, R.M.; Castaño, C.; Adzet, T.; Marin, E.; Parella, T.; Cañigeral, S.; *Planta Med*, **1996,** *62*, 232-235.
[118] Tortoriello, J.; Meckes-Fischer, M.; Villareal, M.L.; Berlin, B.; Berlin, E.; *Phytomedicine*, **1995,** *2*, 57-66.
[119] Rojas, A.; Bah, M.; Rojas, J.I.; Serrano, V.; Pacheco, S.; *Phytomedicine*, **1999,** *6*, 367-371.
[120] Tortoriello, J.; Aguilar-Santamaria, L.; *J. Ethnopharmacol*, **1996,** *53*, 157-163.
[121] Brandao, L.M.; Gamberini, M.T.; Roque, N.F.; Lima-Landman, M.T.; Souccar, C.; Lapa, A.J.; *Phytochemistry*, **2000,** *55*, 617-619.
[122] Gamberini, M.T.; Skorupa, L.A.; Souccar, C.; Lapa, A.J.; *Mem. Inst. Oswaldo Cruz*, **1991,** *86*, 137-139.
[123] Soicke, H.; Leng-Peschlow, E.; *Planta Med*, **1987,** *53*, 37-39.
[124] Pedrazzi, A.H.P.; Rodrigues, E.R.; Zanardo-Filho, A.; Franco, J.J.; Vighnewski, W.; *Fitoterapia*, **1997,** *68*, 26-28.
[125] Bianchi, N.R.; Da Silva, M.O.; Spiassi, L.; Bergonci, J.I.; Machado, C.A.; *Rev. Bras. Farm*, **1993,** *74*, 79-80.
[126] Sobottka, A.M.; Langeloh, A.; Schenkel, E.P.; *Rev. Cienc. Farm*, **1996,** *17*, 147-153.
[127] Vargas, V.M.; Guiodobono, R.R.; Henriques, J.A.; *Memorias do Instituto Oswaldo Cruz*, **1991,** *86*, 67-70.
[128] De Fatima, A.S.M.; De Jesus, N.L.; *Rev. Bras. Farm*, **1996,** *77*, 88-96.
[129] Palazzo de Mello, J.C.; Petrovick, P.R.; *Acta Farm. Bonaerense*, **2000,** *19*, 211-215.
[130] Gianello, J.C.; Ceñal, J.P.; Giordano, O.S.; Tonn, C.E.; Petenatti, M.E.; Petenatti, E.M.; Del Vitto, L.A.; *Acta Farm. Bonaerense*, **2000,** *19*, 99-103.
[131] Jarvis, B.B.; Wang, S.; Cox, C.; Rao, M.M.; Phillip, V.; Varaschin, M.S.; Barros, C.S.; *Nat. Tox*, **1996,** *4*, 58-71.

[132] Rizzo, I.; Varsavky, E.; Haidukowski, M.; Frade, H.; *Toxicon,* **1997,** *35,* 753-757.
[133] Lopez, T.A. ; *Biomed. Environ. Sci,* **1989,** *2,* 7-16.
[134] Mirocha, C.J.; Abbas, H.K.; Treeful, L.; Bean, G.; *Appl. Environ. Microbiol,* **1988,** *54,* 2277-2280.
[135] Bergmann, F.; Yagen, B.; Jarvis, B.B.; *Toxicon,* **1992,** *30,* 1291-1294.
[136] Yagueddu, C.; Cid, M.S.; Lopez, T.; *J. Range Manag,* **1998,** *51,* 655-660.
[137] Telleria, M.C.; *Grana,* **1996,** *35,* 302-307.
[138] Cavelier, J.; Santos, C.; *Rev. Biol. Trop,* **1999,** *47,* 775-784.
[139] Posada, J.M.; Aide, T.M.; Cavelier, J.; *Restoration Ecol,* **2000,** *8,* 370-379.
[140] Mahboubi, P.; Gordon, A.M.; Stoskopf, N.; Voroney, R.P.; *Agroforestry Syst,* **1997,** *37,* 59-77.
[141] Danzer, S.J.; Jemison, R.; Guertin, D.P. ; *Southwestern Naturalist,* **2001,** *46,* 191-199.

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# HOST DEFENSIVE AND PHARMACOLOGICAL STUDY OF *CHLORELLA VULGARIS STRAIN CK*

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**ABSTRACT:** *Chlorella* has been used as a health food for the past 30 years in Japan and in other countries. *Chlorella vulgaris* has been shown to express various pharmacological effects in animals and humans. Hot water extracts of *Chlorella vulgaris* (CVE) are well known to be potent biological modifiers that have immune responses against tumors, bacteria, viruses, and native antigens such as casein. It was reported in 1995 that oral administration of CVE enhances resistance to *Listeria monocytogenes* by augmenting *Listeria*-specific cell-mediated immunity in both normal mice and mice with murine acquired immunodeficiency syndrome (MAIDS) caused by murine leukemia virus *LP-BM5 MuLV.* CVE is thought to augment *Listeria*-specific cell-mediated immunity in both normal and MAIDS mice, in association with increased numbers of $CD4^+$ TCRαβ cells in the infected sites after *L.monocytogenes* infection. In our previous report in 1999, oral administration of CVE also inhibited IgE production against casein with an impaired Th2 response. In the field of environmental science, *Chlorella vulgaris strain CK* has shown to accelerate dioxin excretion in rats. The fecal excretions of dioxins were greater in rats fed *Chlorella.* These findings suggested that the administration of *Chlorella* may be useful in preventing gastrointestinal absorption and in promoting the excretion of dioxin already absorbed in tissues. As well, studies of γ-aminobutyric acid (GABA)-rich *Chlorella* and docosahexaenoic acid (DHA)-rich *Chlorella* between 2000 and 2002 were reviewed. γ-aminobutyric acid (GABA) is known to exert a hypotensive effect on blood pressure. A method has been established for producing large amounts of GABA in *Chlorella vulgaris strain CK.* It has been reported that the GABA-rich *Chlorella* showed a significant hypotensive effect in spontaneously hypertensive rats (SHR) and humans. Docosahexaenoic acid (DHA) has been shown to exert protective effects via numerous physiological actions. In order to produce DHA-rich *Chlorella* cells, *Chlorella vulgaris strain CK* was heterotrophycally grown in the pilot scale fermentor. The exogenous DHA was taken up and accumulated in *Chlorella* cells. Next, the effect of docosahexaenoic (DHA)-rich *Chlorella vulgaris* on hyperlipidemia has been studied in rats. An anti-hyperlipidemic effect was found in rats fed DHA-rich *Chlorella vulgaris.*

Thus, the benefits of the *Chlorella* organism are shown to be very wide-ranging, and future studies can be expected regarding the nutritional fortification of *Chlorella* by adding functional substances to *Chlorella* cells.

# INTRODUCTION

## I. An overview of pharmacological profiles of green algae, *Chlorella*, in regard to detoxification and cancer prevention in the last ten years.

### What is *Chlorella* ?

*Chlorella* is the unicellular green algae that has been widely used for biochemical and physiological studies. Its cells are small and structurally quite simple, containing a relatively large cup-shaped chloroplast for photosynthesis and a tiny nucleus. A single cell of *Chlorella* can divide into four cells every 16 h to 20 h, utilizing sunlight for photosynthesis.

*Chlorella* contains protein, chlorophyll, dietary fiber, and large amounts of minerals and vitamins. The protein of *Chlorella* contains all the essential amino acids required for the nutrition of animals and humans.

### Protection against a chemical-induced cancer by *Chlorella vulgaris* administration

Oral administration of *Chlorella vulgaris* in regard to colorectal carcinomas induced by 1, 2-dimethylhydrazine (DMH) was examined in a rat model [1]. DMH was injected subcutaneously 20 mg/kg body weight once a week for 10 weeks. After 30 weeks, the incidence and total number of colorectal cancers were investigated. The result showed that *Chlorella vulgaris* administration was markedly protective against the development of colorectal carcinomas.

In addition, the effect of *Chlorella vulgaris* administration on skin papillomas induced by 7, 12-dimethylbenzantracene (DMBA) was examined in mice [2]. Topical application of *Chlorella vulgaris* modulated the percent incidence of mice skin papillomas in the initiation stages of carcinogenesis by DMBA.

### *Chlorella vulgaris* accelerates dioxin excretion

Environmental contaminants have already led to detectable levels of these industrial toxins in humans and animals. Many toxic chemicals such as dioxin and polychlorinated biphenyl (PCB) are not readily detoxified or excreted into feces. We investigated the effects of *Chlorella vulgaris strain*

*CK* on the fecal excretion of polychlorinated dibenzo-*p*-dioxin (PCDD) congeners and polychlorinated dibenzofuran (PCDF) congeners in rats administered the dioxin mixture [3].

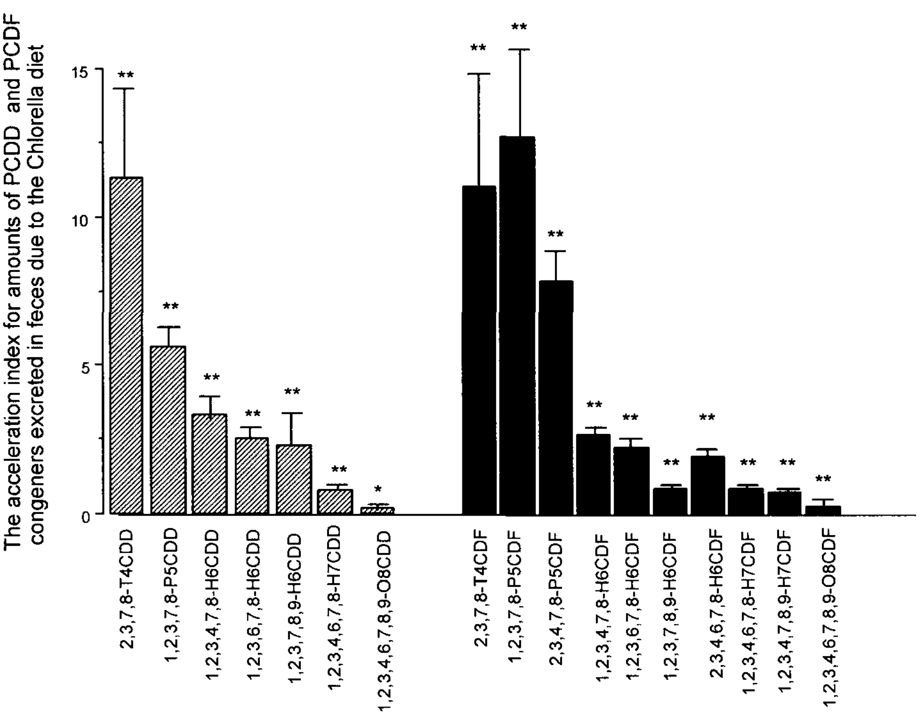


Figure 1 Effects of *Chlorella* diet on fecal excretion amounts of polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners in contaminated rice oil-fed rats. Values are means±SD, n=4. Values of acceleration index of fecal excretion amounts of PCDD and PCDF congeners due to *Chlorella* diet were calculated using the following equation: Acceleration index of fecal excretion amounts of PCDD and PCDF congeners due to *Chlorella* diet = {(percent fecal excretion in the *Chlorella* group) / (percent fecal excretion in the control group) - 1}. Significant difference from the control diet, *: $P < 0.05$, **:$P < 0.01$.

The results showed that the amounts of PCDD and PCDF congeners excreted into feces from d1 to d5 in the group fed the 10 % *Chlorella* diet were 0.2-11.3 and 0.3-12.8 times greater ($P<0.05$) than those of the control group, respectively (Fig.(**1**)). In rats fed the 10 % *Chlorella* diet, the excretion of 2, 3, 7, 8- tetrachlorodibenzo-*p*-dioxin ($T_4CDD$) was significantly greater than that of highly chlorinated PCDD congeners, and the excretions of 2, 3, 7, 8-tetrachlorodibenzofuran ($T_4CDF$), 1, 2, 3, 7, 8-pentachlorodibenzofuran ($P_5CDF$), and 2, 3, 4, 7, 8-$P_5CDF$ were significantly greater than those of highly chlorinated PCDF congeners.

In this study, highly toxic PCDD and PCDF congeners were more easily

excreted into the feces than weakly toxic, highly chlorinated PCDD and PCDF congeners due to *Chlorella* administration.

*Chlorella* and green vegetables contain large amounts of chlorophyll. The most effective compound in *Chlorella* cells and green vegetables for promoting the fecal excretion of dioxin is likely to be chlorophyll.

Table 1 Effect of the chlorophyll diet on fecal excretion of PCDD and PCDF congeners in rats administered the dioxin mixture.

| Dioxins | | Chlorophyll diet (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ng TEQ | 0 (Basal diet) | 0.01 | 0.02 | 0.05 | 0.1 | 0.2 | 0.5 |
| 2,3,7,8-T4CDD | 10 | 0.43±0.14 | 0.67±0.17(155.8) | 0.74±0.15 (172.1)b | 0.99±0.20(230.2)a | 1.69±0.51(393.0)a | 3.90±1.67(907.0)a | 5.87±2.48(1365.1)a |
| 1,2,3,7,8-P5CDD | 10 | 1.05±0.28 | 1.73±0.12 (164.8)a | 2.18±0.25(207.6)a | 4.14±0.77(394.3)a | 6.14±1.50(584.8)a | 9.60±2.31(914.3)a | 13.11±2.09(1248.6)a |
| 1,2,3,4,7,8-H6CDD | 1 | 0.28±0.06 | 0.50±0.06 (178.6)a | 0.69±0.04(246.4)a | 0.95±0.13(339.3)a | 1.42±0.18(507.1)a | 1.68±0.21(600.0)a | 1.95±0.19(696.4)a |
| 1,2,3,6,7,8-H6CDD | 1 | 0.34±0.07 | 0.59±0.07 (173.5)a | 0.78±0.05(229.4)a | 1.08±0.15(317.6)a | 1.60±0.17(470.6)a | 1.95±0.22(573.5)a | 2.39±0.12(702.9)a |
| 1,2,3,7,8,9-H6CDD | 1 | 0.65±0.09 | 1.03±0.12(158.5)a | 1.43±0.04 (220.0)a | 1.71±0.21(263.1)a | 1.98±0.13(304.6)a | 2.09±0.19(321.5)a | 2.41±0.10(370.8)a |
| 1,2,3,4,6,7,8-H7CDD | 0.1 | 0.12±0.01 | 0.16±0.01(133.3)a | 0.20±0.01 (166.7)a | 0.22±0.03(183.3)a | 0.25±0.02(208.3)a | 0.27±0.02(225.0)a | 0.27±0.01(225.0)a |
| 1,2,3,4,6,7,8,9-O8CDD | 0.002 | 0.0043±0.0002 | 0.0045±0.0003(104.7) | 0.0057±0.0003 (132.6)a | 0.0052±0.0008(120.9) | 0.0050±0.0003(116.3) | 0.0051±0.0003(118.6) | 0.0049±0.0004(114.0)a |
| 2,3,7,8-T4CDF | 1 | 0.03±0.01 | 0.05±0.01(166.7) | 0.05± 0.01(166.7) | 0.08±0.02 (266.7)a | 0.11±0.04 (366.7)a | 0.29±0.17(966.7)a | 0.52±0.23(1773.3)a |
| 1,2,3,7,8-P5CDF | 0.5 | 0.07±0.02 | 0.13±0.02 (185.7)a | 0.19±0.02 (271.4)a | 0.26±0.05(371.4)a | 0.43±0.08(614.3)a | 0.62±0.11(885.7)a | 0.84±0.12(1200.0)a |
| 2,3,4,7,8-P5CDF | 5 | 0.44±0.13 | 0.78±0.08(177.3)a | 1.00±0.14(227.3)a | 1.81±0.39(411.4)a | 2.86±0.69(650.0)a | 4.76±1.30(1081.8)a | 6.91±1.44(1570.5)a |
| 1,2,3,4,7,8-H6CDF | 1 | 0.44±0.07 | 0.83±0.11(188.6)a | 1.09±0.05(247.7)a | 1.45±0.15(329.5)a | 1.86±0.09(422.7)a | 2.07±0.21(470.5)a | 2.33±0.19(529.5)a |
| 1,2,3,6,7,8-H6CDF | 1 | 0.41±0.06 | 0.73±0.08(178.0)a | 0.98±0.05(239.0)a | 1.38±0.10(336.6)a | 1.65±0.15(402.4)a | 2.09±0.27(509.8)a | 2.27±0.13(553.7)a |
| 1,2,3,7,8,9-H6CDF | 1 | 0.40±0.06 | 0.65±0.07(162.5)a | 0.88±0.04(220.0)a | 1.25±0.09(312.5)a | 1.57±0.15(392.5)a | 1.86±0.24(465.0)a | 2.17±0.21(542.5)a |
| 2,3,4,6,7,8-H6CDF | 1 | 0.44±0.06 | 0.81±0.09(184.1)a | 1.11±0.03(252.3)a | 1.47±0.12(334.1)a | 1.76±0.15(400.0)a | 2.05±0.20(465.9)a | 2.27±0.16(515.9)a |
| 1,2,3,4,6,7,8-H7CDF | 0.1 | 0.12±0.01 | 0.17±0.01(141.7)a | 0.21±0.01(175.0)a | 0.22±0.01(183.3)a | 0.23±0.01(191.7)a | 0.24±0.01(200.0)a | 0.26±0.02(216.7)a |
| 1,2,3,4,7,8,9-H7CDF | 0.1 | 0.10±0.01 | 0.14±0.01(140.0)a | 0.17±0.00(170.0)a | 0.19±0.02(190.0)a | 0.23±0.02(230.0)a | 0.25±0.02(250.0)a | 0.27±0.01(270.0)a |
| 1,2,3,4,6,7,8,9-O8CDF | 0.002 | 0.0039±0.0002 | 0.0046±0.0004(117.9) | 0.0049±0.0001(125.6)a | 0.0050±0.0005(128.2)a | 0.0052±0.0001(133.3)a | 0.0052±0.0003(133.3)a | 0.0055±0.0001(141.0)a |
| Total | 33.804 | 5.32±1.01 | 8.97±0.48(168.6)a | 11.69±0.56(219.7)a | 17.22±2.38(323.7)a | 23.77±3.78(446.8)a | 33.71±6.93(633.6)a | 43.83±6.63(823.9)a |

Values represent the mean±SD (n=4)
Values represent the percent fecal excretion of PCDD and PCDF congeners from d 1 to d 5 against total ng of TEQ values in rats administered PCDD and PCDF congeners.
The acceleration index of fecal excretion (the percent fecal excretion of PCDD and PCDF congeners of rats in the chlorophyll diet / in the control diet x 100) are in parentheses.
a:Significantly different from basal group ($p<0.01$). b:Significantly different from basal group ($p<0.05$).

As shown in Table 1, the amounts of fecal excretion of PCDD and PCDF congeners in rats fed the chlorophyll diet were greater than those of rats fed the control diet in a dose-dependent manner [4]. The preferential effects of chlorophyll on low toxic equivalents (TEQ) compounds such as 1, 2, 3, 4, 6, 7, 8, 9-octachlorodibenzo-*p*-dioxin ($O_8CDD$) and 1, 2, 3, 4, 6, 7, 8, 9-octachlorodibenzofuran ($O_8CDF$) may indicate a less effective detoxication than high TEQ compounds such as 2, 3, 7, 8-$T_4CDD$; 1, 2, 3, 7, 8-$P_5CDD$ and 2, 3, 4, 7, 8-$P_5CDF$. We found the amounts of fecal excretion of 2, 3, 7, 8-$T_4CDD$; 1, 2, 3, 7, 8-$P_5CDD$, and 2, 3, 4, 7, 8-$P_5CDF$, which are highly toxic, in rats fed the 0.2 % chlorophyll diet were 807 %, 814 %, and 982 % greater than those of the control group, respectively.

These results are critical for evaluating the efficancy of chlorophyll as an agent for decreasing intake of toxic PCDD and PCDF congeners. Thus, chlorophyll administration enhanced the fecal excretion of PCDD and PCDF congeners and reduced PCDD and PCDF congeners absorption in rats administered the dioxin mixture.

Table 2 Effect of chlorophyll diet on body burden of PCDD and PCDF congeners in rats administered the dioxin mixture

| | | Chlorophyll diet (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dioxins | ng TEQ | 0 (Basal diet) | 0.01 | 0.02 | 0.05 | 0.1 | 0.2 | 0.5 |
| 2,3,7,8-T$_4$CDD | 10 | 27.52±0.81 | 26.57±1.67(96.5) | 26.67±0.44 (96.9) | 26.16±1.59(95.1) | 25.46±1.26(92.5)[b] | 24.09±2.52(87.5)[b] | 22.31±1.69(81.1)[a] |
| 1,2,3,7,8-P$_5$CDD | 10 | 27.86±2.64 | 25.69±2.32 (92.2) | 25.13±1.27(90.2) | 22.21±1.53(79.7)[a] | 19.93±1.17(71.5)[a] | 17.77±1.84(63.8)[a] | 14.19±2.57(50.9)[a] |
| 1,2,3,4,7,8-H$_6$CDD | 1 | 2.65±0.22 | 2.32±0.16 (87.5) | 2.22±0.04(83.8)[a] | 1.75±0.18(66.0)[a] | 1.37±0.19(51.7)[a] | 1.01±0.19(38.1)[a] | 0.71±0.19(26.8)[a] |
| 1,2,3,6,7,8-H$_6$CDD | 1 | 2.50±0.08 | 2.15±0.10 (86.0)[a] | 1.98±0.05(79.2)[a] | 1.65±0.22(66.0)[a] | 1.32±0.16(52.8)[a] | 0.96±0.15(38.4)[a] | 0.62±0.11(24.8)[a] |
| 1,2,3,7,8,9-H$_6$CDD | 1 | 2.23±0.23 | 1.79±0.16(80.3)[b] | 1.51±0.08 (67.7)[a] | 1.05±0.09(47.1)[a] | 0.81±0.15(36.3)[a] | 0.63±0.19(28.3)[a] | 0.41±0.13(18.4)[a] |
| 1,2,3,4,6,7,8-H$_7$CDD | 0.1 | 0.17±0.01 | 0.11±0.02(64.7)[a] | 0.08±0.00(47.1)[a] | 0.05±0.01(29.4)[a] | 0.04±0.01(23.5)[a] | 0.03±0.01(17.6)[a] | 0.02±0.01(11.8)[a] |
| 1,2,3,4,6,7,8,9-O$_8$CDD | 0.002 | 0.0014±0.0002 | 0.0007±0.0001(50.0) | 0.0005±0.0001 (35.7) | 0.0003±0.0001(21.4)[b] | 0.0004±0.0001(28.6)[a] | 0.0004±0.0003(28.6)[a] | 0.0002±0.0001(14.3)[a] |
| 2,3,7,8-T$_4$CDF | 1 | 0.73±0.16 | 0.76±0.11(104.1) | 0.83±0.16(113.7) | 0.90±0.16 (123.3) | 0.73±0.08 (100.0) | 0.94±0.20(128.8) | 0.93±0.12(127.4) |
| 1,2,3,7,8-P$_5$CDF | 0.5 | 0.73±0.16 | 0.59±0.14 (80.8) | 0.66±0.11 (90.4) | 0.52±0.11(71.2) | 0.48±0.13(65.8) | 0.47±0.14(64.4) | 0.33±0.10(45.2)[a] |
| 2,3,4,7,8-P$_5$CDF | 5 | 14.14±0.83 | 13.62±0.29(96.3) | 12.54±0.58 (88.7)[b] | 11.25±1.03(79.6)[a] | 10.57±0.66(74.8)[a] | 9.31±1.07(65.8)[a] | 7.61±0.96(53.8)[a] |
| 1,2,3,4,7,8-H$_6$CDF | 1 | 2.55±0.15 | 2.04±0.13(80.0)[a] | 1.81±0.05(71.0)[a] | 1.23±0.13(48.2)[a] | 1.01±0.12(39.6)[a] | 0.69±0.14(27.1)[a] | 0.44±0.12(17.3)[a] |
| 1,2,3,6,7,8-H$_6$CDF | 1 | 2.47±0.19 | 2.09±0.12(84.6)[b] | 1.88±0.10(76.1)[a] | 1.40±0.12(56.7)[a] | 1.08±0.17(43.7)[a] | 0.80±0.19(32.4)[a] | 0.51±0.13(20.6)[a] |
| 1,2,3,7,8,9-H$_6$CDF | 1 | 2.16±0.28 | 1.85±0.21(85.6) | 1.70±0.16(78.7)[b] | 1.21±0.13(56.0)[a] | 1.02±0.18(47.2)[a] | 0.78±0.20(36.1)[a] | 0.48±0.13(22.2)[a] |
| 2,3,4,6,7,8-H$_6$CDF | 1 | 2.41±0.16 | 2.13±0.17(88.4) | 1.76±0.05(73.0)[a] | 1.20±0.14(49.8)[a] | 0.99±0.15(41.1)[a] | 0.73±0.16(30.3)[a] | 0.46±0.13(19.1)[a] |
| 1,2,3,4,6,7,8-H$_7$CDF | 0.1 | 0.13±0.02 | 0.08±0.01(61.5)[a] | 0.06±0.00(46.2)[a] | 0.04±0.01(30.8)[a] | 0.03±0.01(23.1)[a] | 0.02±0.01(15.4)[a] | 0.01±0.01(7.7)[a] |
| 1,2,3,4,7,8,9-H$_7$CDF | 0.1 | 0.19±0.01 | 0.13±0.01(68.4)[a] | 0.11±0.00(57.9)[a] | 0.07±0.01(36.8)[a] | 0.05±0.01(26.3)[a] | 0.04±0.02(21.1)[a] | 0.02±0.01(10.5)[a] |
| 1,2,3,4,6,7,8,9-O$_8$CDF | 0.002 | 0.0012±0.0002 | 0.0007±0.0001(58.3) | 0.0005±0.0000(41.7) | 0.0003±0.0002(25.0)[a] | 0.0004±0.0001(33.3)[a] | 0.0003±0.0003(25.0)[b] | 0.0001±0.0001(8.3)[a] |
| Total | 33.804 | 88.44±5.33 | 81.91±3.86(92.6) | 78.91±2.55(89.2)[b] | 70.68±3.73(79.9)[a] | 64.88±3.86(73.4)[a] | 58.25±6.41(65.9)[a] | 49.04±5.84(55.5)[a] |

Values represent the mean±SD, n=4.
Significantly different from basal group (a, $p<0.01$; b, $p<0.05$).
Each value represents percent body burden of PCDD and PCDF congeners against total ng of TEQ values in rats administered PCDD and PCDF congeners.
Acceleration index of disappearance from the body (shown in parentheses) equals (percent body burden of PCDD and PCDF congeners of rats in the chlorophyll diet) / (percent body burden of PCDD and PCDF congeners of rats fed the control diet ) x 100.

The amounts of body burden of PCDD and PCDF congeners in rats fed the chlorophyll diet were lower in a dose-dependent manner than those of rats fed the control diet (Table 2). The preferential effects of chlorophyll on low TEQ compounds such as 1, 2, 3, 4, 6, 7, 8, 9-O$_8$CDD and 1, 2, 3, 4, 6, 7, 8, 9-O$_8$CDF may indicate a less effective detoxification than high TEQ compounds such as 2, 3, 7, 8-T$_4$CDD, 1, 2, 3, 7, 8-P$_5$CDD, and 2, 3, 4, 7, 8-P$_5$CDF. These results are critical for evaluating the efficacy of chlorophyll as an agent for decreasing the intake of toxic PCDD and PCDF congeners.

*Chlorella* and green vegetables contain large amounts of chlorophyll. As stated above, the most effective compound in *Chlorella* cells and green vegetables for promoting the fecal excretion of dioxin is likely to be chlorophyll. It was reported that chlorophyllin, a chlorophyll derivative, formed complexes with heterocyclic amines and exhibited potent antimutagenic activity [5]. Chlorophyllin may form a complex with dioxins having a planar structure, leading to reduced biotoxicity. It was reported that the chlorophyll from vegetables inhibited cytochrome P450-dependent monooxygenases [6].

Eating foods such as *Chlorella*, green vegetables, and seaweeds containing chlorophyll seems to protect against health disorders caused by dioxin exposure in humans by capturing dioxin in the digestive tract and by diminishing the amount of dioxin absorption.

## Accelerated detoxicating enzyme activity by *Chlorella* administration

Pore reported that *Chlorella protothecoides* accelerated the detoxication of chlordecone-poisoned rats, decreasing the half-life of the toxin from 40 to 19 days [7]. *Chlorella vulgaris* induced a significant increase in the hepatic levels of glutathione S-transferase and sulfhydryl (-SH) over a 14-day treatment in gestating or lactating mice [8]. *Chlorella vulgaris* modulated the inhibition of cytochrome b5, cytochrome P-450, and malondialdehyde levels in the fetal and neonatal hepatic tissue following transplacental or translactational exposure.

These modulations suggest that *Chlorella vulgaris* potentiates chemopreventive action via the perinatal passage of active constituents and/or metabolites. Moreover, *Chlorella vulgaris* is capable of protecting haemopoietic tissue of whole body-irradiated mice [9]. This protection is seen both in terms of haemopoietic stem cells and the survival of animals.

## II. An overview of the immunopharmacology of green algae, *Chlorella* in the last ten years

### Augmentation of the host defense against *Listeria* infection with normal or murine leukemia viruses, *LP-BM5 MuLV* infected-immunocompromissed mice by hot water extracts (CVE) administration

We have shown that oral administration with hot water extracts (CVE) significantly augmented the host defense against infection with *Listeria monocytogenes (L.monocytogenes)* [10]. *L.monocytogenes* is one of an intracellular bacteria, as well as Mycobacterium tuberculosis [11-12]. The protective mechanism against *Listeria* infection depends mainly on the immune response mediated by $\alpha\beta$ T cells [13].

We have recently reported that $\gamma\delta$ T cells precede $\alpha\beta$ T cells in appearance in the peritoneal cavity during *Listeria* infection and play an important role in protection at the early stage after listerial infection [14-16]. We found that CVE augmented the host defense against *Listeria* infection by augmenting cell-mediated immunity at the early stage after infection.

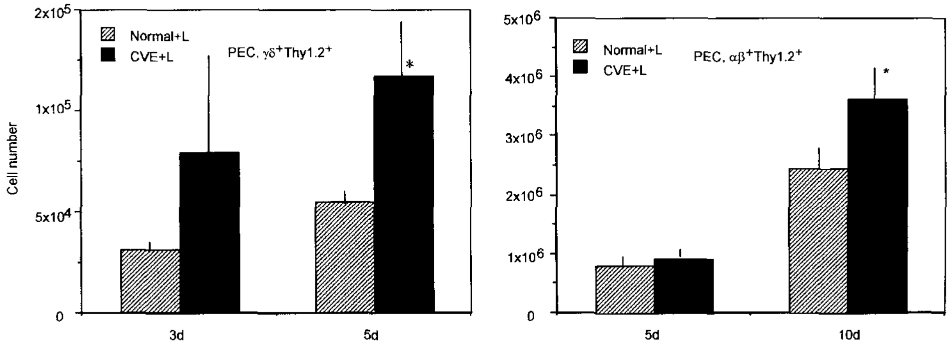


Figure 2. γδ T cells in nonadherent PEC obtained from CVE-administered mice after i.p. inoculation with *L.monocytogenes*. Nonadherent PEC obtained various times after infection with *L.monocytogenes* were stained with anti-TCRαβ, anti-PE-Thy1.2, followed by goat anti-hamstar FITC-IgG, or anti-Bio-TCRγδ, anti-PE-Thy1.2, followed by Streptavidin FITC. *: Significantly different from the value for the control group, $P<0.05$).

The FCM analysis using mAbs to Thy1.2 and γδ TCR was carried out on the nonadherent population of the PEC in normal mice and CVE-administered mice at the early stage after an i.p. infection with *L.monocytogenes* (Fig.(**2**)). Consistent with our previous reports, γδ T cells increased in the PEC of control mice on day 3 and on day 5 after *Listeria* infection. The increment of the proportion of γδ T cells in the PEC of CVE-administered mice infected with *L.monocytogenes* was significantly higher than that seen in untreated control mice.

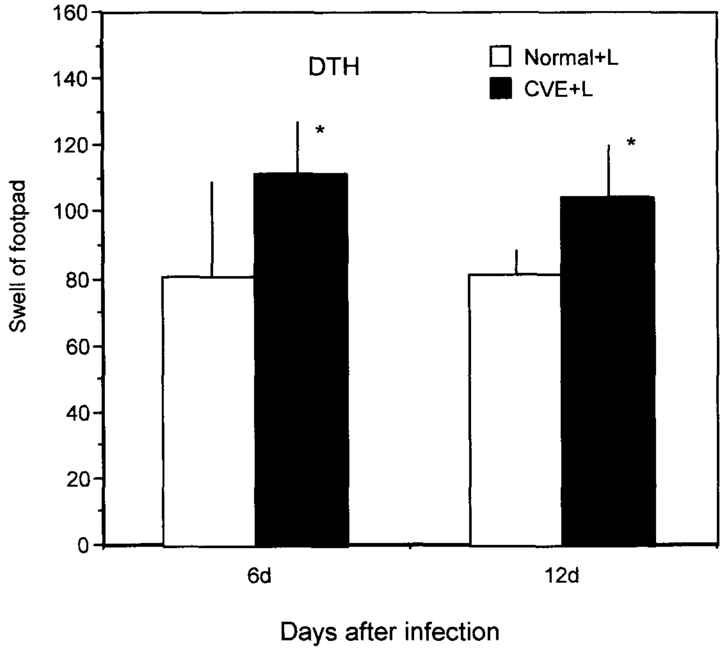

Figure 3. The kinetics of DTH responses of CVE-administered mice infected with *L.monocytogenes*. The DTH response was determined at 6 or 12 days after an i.p. injection of a sublethal dose ($1x10^4$/a) of *L.monocytogenes*. Mice were injected $1.6x10^8$ heat-killed *Listeria* cells in 50 μl of pyrogen-free PBS into the right footpad. The left footpad of each mouse received an equal volume of PBS. Twenty-four hours later the thickness of each footpad was measured using a dialgauge micrometer. Data values are expressed as means±standard deviation (n=6). *: Significantly different from the value for the control group, P<0.05).

In order to compare the development of *Listeria*-specific cell-mediated immunity between the CVE-administered group and the control group, we examined DTH response using heat-killed *L.monocytogenes* as the eliciting antigens. As shown in Figure (**3**), the DTH response of CVE-administered mice in the early stage of infection on day 6 was significantly higher than that of the control mice. Afterward, DTH response of CVE-administered mice maintained a constant level on day 12 after infection. These results suggested that CVE administration effectively augment cell-mediated immunity in correlation with an increase of γδ T cells at the early phase of *Listeria* infection. CVE administration augmented DTH response from the early stage to the late stage after infection.

Increase in number of $CD4^+$ T cells in CVE-administered mice at the late phase after infection may be due mainly to Th1 cells (data not shown). Alternatively, the increment of DTH response seems to be closely correlated with an increase of γδ T cells in CVE-administered mice at the early phase of infection. It is suggested that γδ T cells may be directly or indirectly associated with the strong DTH response appearing at the early stage after infection.

CVE has been shown to be a potent host-mediated immuno-modulator by augmenting the production of a cytokine/cell growth factor, such as GM-CSF, and/or the responsiveness of the progenitor to CSF [17]. In conclusion, oral administration of CVE augments the resistance against listerial infection via the early development of cell-mediated immunity to *Listeria*.

Murine leukemia virus *LP-BM5 MuLV* is known to induce a remarkable immunodeficiency syndrome in C57BL/6 mice [18]. The syndrome caused by this virus is called murine acquired immunodeficiency syndrome (MAIDS). This virus also causes an early selective defect in $CD4^+$ Th-cell function, and some whose symptoms are similar to those found in AIDS patients [19]. MAIDS mice are a useful model for elucidating the mechanism of depressed resistance to opportunistic pathogens common in AIDS patients [20-21].

The therapeutic potential of CVE against *Listeria* resistance in an immunocompromised host has been evaluated [22]. In this report, we

found that the protection against the late phase of *Listeria* infection is impaired in MAIDS mice, and the numbers of bacteria in target organs in CVE-treated MAIDS mice were lower than those in MAIDS mice at the late phase after *Listeria* infection (Fig. (**4**)).

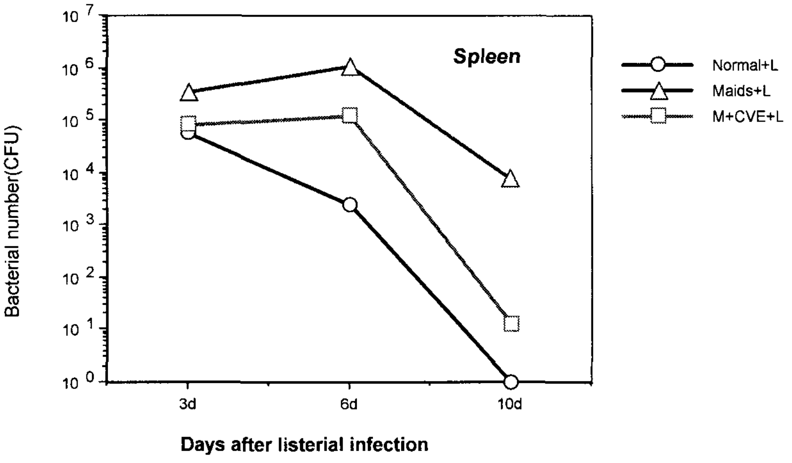


**Figure 4**

Effect of oral administration of CVE on the growth of *L.monocytogenes* in the spleen of *LP-BM5*-infected mice. Mice were infected with 0.1ml of *LP-BM5* intraperitoneally and were challenged with *L.monocytogenes* ($2x10^4$) on week 4 after *LP-BM5* infection. Two percent CVE-containing solid chows were given to the mice infected with *LP-BM5* on week 2 and were given until the experiments were over. Data values are representative of two experiments (n=5). *: Significantly different from the value for the control group, $P<0.05$.

The listerial number in target organs were eliminated completely in CVE-treated MAIDS mice on day 10 after listerial infection, whereas *Listeria* in the spleen and peritoneal cavity were still detectable in MAIDS mice on day 10 after listerial infection. Thus, the difference between CVE-treated MAIDS mice and MAIDS mice was most noticeable on day 10 after listerial infection.

It has been reported that DTH response results in swelling and a predominantly mononuclear cell infiltration at the injection site [23].

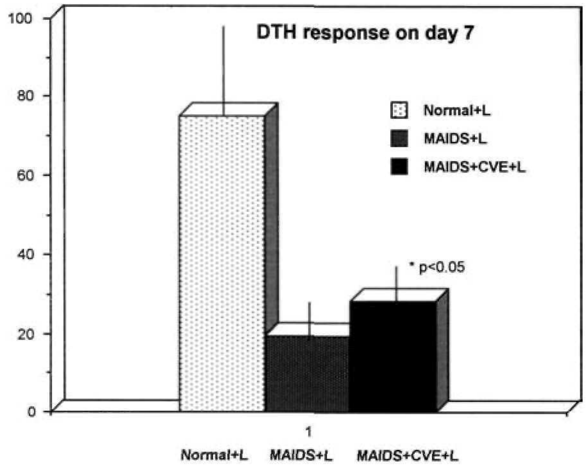


**Figure 5** The kinetics of DTH responses of CVE-administered mice infected with *L.monocytogenes*. Mice were infected with 0.1ml of *LP-BM5* intraperitoneally and were challenged with *L.monocytogenes* ($2x10^4$) on week 4. Two percent CVE-containing solid chows were given to the mice infected with *LP-BM5* on week 2 and were given until the experiments were over. The DTH response was determined on day 7 after an injection of sublethal dose ($2x10^4$) of *L.monocytogenes*. Mice were injected $1.6x10^8$ heat-killed *Listeria* cells in 50 μl of pyrogen-free PBS into right footpad. The left footpad of each mouse received an equal volume of PBS. Twenty-four hours later, the thickness of each footpad was measured using a dialgauge micrometer. Data values are representative of two experiments and are expressed as means±standard deviation (n=8). *: Significantly different from the value for the control group, $P<0.05$.

The DTH response to *Listeria* antigen in CVE-treated MAIDS mice was significantly higher than that of MAIDS mice on day 7 after *Listeria* infection, although the response was much lower than in normal mice.

DTH response can be partially ascribed to the production of IFNγ by Thl cells in response to the antigen [23]. IL-12 released from macrophages is reported to stimulate NK and Thl cells to produce IFNγ [24]. We speculated that CVE administration augments IFNγ secretion through the activation of macrophages to produce several monokines including IL-12 and to consequently restore the response to the presentation of *Listeria*-specific antigens.

Next, in order to elucidate the mechanism whereby CVE augments the immune response against *L.monocytogenes*, we examined the effects of oral administration of CVE on the expression of mRNA specific for various cytokines in the adherent cells of normal mice and MAIDS mice infected with *L.monocytogenes*.

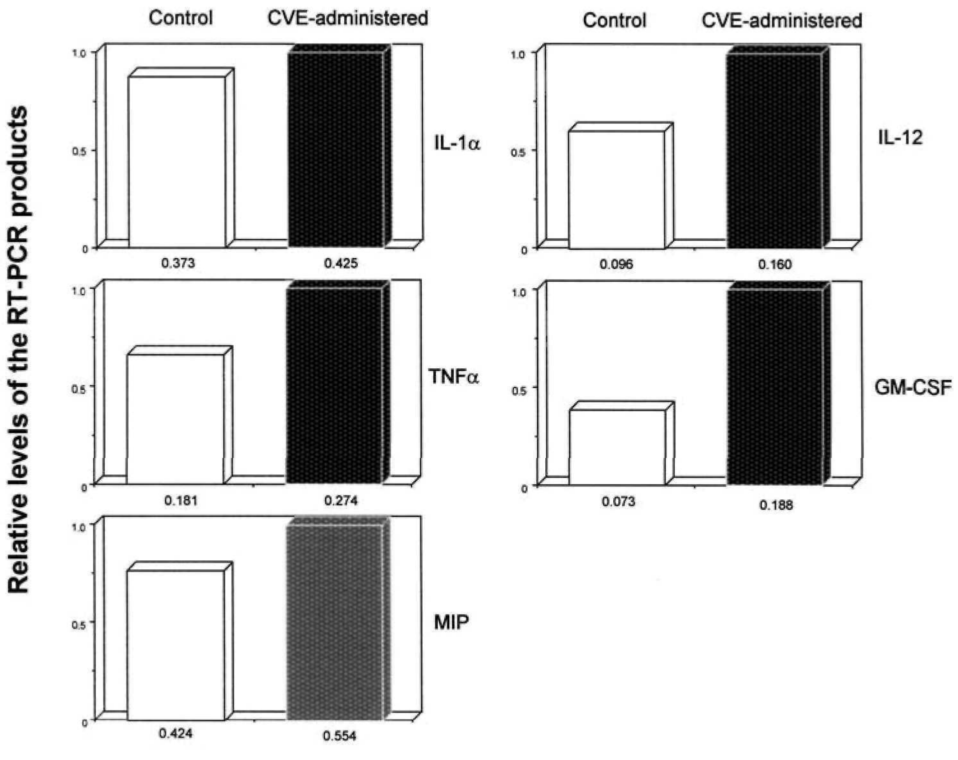


Figure 6 RT-PCR analysis of cytokine mRNA expression in the adherent PEC of mice given CVE. Two percent CVE-containing solid chows were given to mice two weeks previously. Total RNAs were extracted from adherent PEC and reverse-transcribed, and the cDNA concentration was adjusted by co-amplification of twofold serially diluted cDNA and constant amounts of control plasmid *pMCQ* with β-actin-specific primers. Cytokine mRNA expression patterns of IL-1α, TNFα, MIP, IL-12 and GM-CSF were determined by PCR using murine cytokine-specific primers, respectively. The PCR products were separated in 2 % agarose gel and visualized by ethidium bromide staining. Relative quantification of the amounts of the RT-PCR products was performed using a Computing Densitometer and MasterScan™software. The signal intensity is presented as the relative value to the maximal optical density (OD) of each cytokine. The value at the bottom of each column indicates net OD measured by a Computing Densitometer and MasterScan™ software.

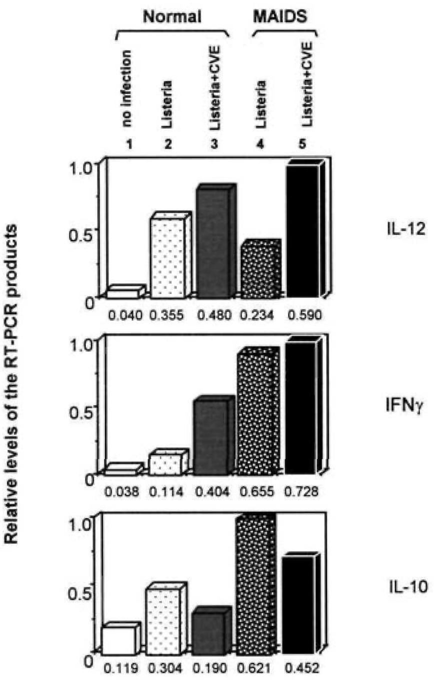


Figure 7 RT-PCR analysis of cytokine mRNA expression in the spleens of normal mice or MAIDS mice infected with *L monocytogenes*. Two percent of CVE-containing solid chows were given to the normal mice or mice infected with *LP-BM5* two weeks previously and 2 weeks later, $2x10^4$ *L.monocytogenes* were inoculated i.p. into both mice groups. Total RNAs were extracted from spleens of the mice of both groups on day 6 after *Listeria* infection using RNAzol™ and reverse-transcribed, and the cDNA concentration was adjusted by co-amplification of twofold serially diluted cDNA and constant amounts of control plasmid *pMCQ* with β-actin-specific primers. Cytokine expression patterns of IL-12, IFNγ, and IL-10 were determined by PCR using murine cytokine-specific primers, respectively. Relative quantification of the amounts of the RT-PCR products was performed using a Computing Densitometer and MasterScan™ software. The signal intensity is presented as the relative value to the maximal OD of each cytokine. The value at the bottom of each column indicates net OD measured using a Computing Densitometer and MasterScan™ software.

1, uninfected mice; 2, mice infected with *L.monocytogenes*; 3, mice given CVE and infected with *L.monocytogenes*; 4, MAIDS mice infected with *L.monocytogenes*; 5, MAIDS mice given CVE and infected with *L.monocytogenes*.

The results were as follows:

(1) Significant increases in expressions of IL-12 and TNFα mRNAs were detected in the peritoneal adherent cells in normal mice after oral administration of CVE.
(2) The expression levels of IL-12 and IFNγ mRNAs were augmented in the spleen of normal mice after infection with *L.monocytogenes* by

CVE administration.

(3) Oral administration of CVE increased the expression levels of IL-12 and IFNγ mRNAs but rather reduced the IL-10 mRNA level in the spleen of MAIDS mice after infection with *L.monocytogenes.*

In conclusion, we demonstrated that oral administration of CVE was related to the retention of the capacity of MAIDS mice to eliminate *L.monocytogenes* in association with an improvement in the defective immune response to *L.monocytogenes.* CVE treatment enhances the host defense against opportunistic *Listeria* infection in immunodeficient mice. Furthermore, we have obtained evidence that oral administration of CVE augments the levels of IL-1α, TNFα and IL-12 mRNA expressions in macrophages. It also augmented the levels of IFNγ mRNA in the spleen of normal mice and MAIDS mice after infection with *Listeria.* CVE may induce Th1-biased immune responses via activation of macrophages and enhance the resistance to *L.monocytogenes* infection in normal and MAIDS mice.

## Inhibition of IgE production against casein by CVE administration

Allergic reactions are characterized by the production of allergen-specific IgE with a predominant Th2 response [25-26]. Helper T cells can be divided largely into two subgroups, Th1 and Th2, on the basis of their cytokine production patterns. Th1 cells, which produce IL-2 and IFNγ, induce cell-mediated immunity characterized by macrophage activation and cytotoxic T lymphocyte (CTL) induction, while Th2 cells, which produce IL-4, IL-5, IL-6, and IL-10, help for antibody production.

Upon exposure to an antigen, activated $CD4^+$ T cells can develop into either Th1 cells, capable of inducing cell-mediated immunity characterized by macrophage activation and CTL induction, or Th2 cells, which induce humoral immunity via B-cell development [27]. $CD4^+$ T cells initially stimulated in the presence of IL-12 and IFNγ tend to develop into Th1 cells. On the other hand, IL-4 promotes the differentiation of Th2 cells and inhibits the generation of Th1 cells. IFNγ, a Th1 type cytokine, promotes Ig class switching to IgG2a, whereas IL-4, a Th2 type cytokine, promotes IgG1 and IgE switching [28].

Next, we tested the ability of CVE to abrogate the antigen-specific response in mice injected i.p. with milk casein in CFA [29].
The level of casein-specific IgE at 3 weeks after immunization was significantly lower in the CVE administered group than in the control group given commercial diet (Fig.(**8**)). In contrast, the level of casein-specific IgG2a at 3 weeks was significantly higher in the CVE administrated group than in the control group (Fig.(**8**)).

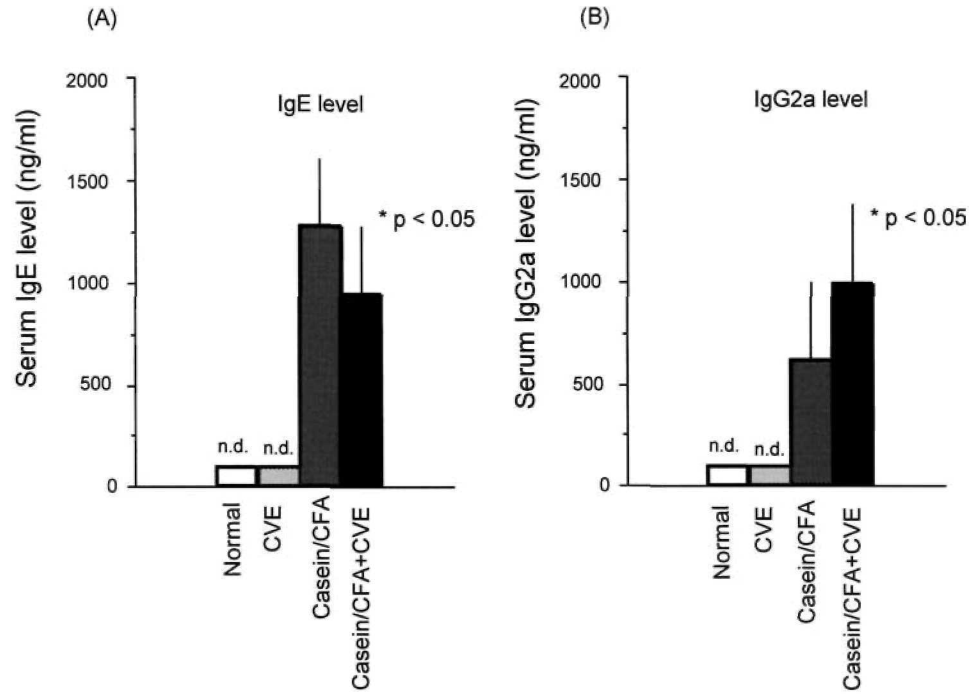


Figure 8 Casein-specific IgE levels in serum from DBA/2 mice which were immunized i.p. with 100 μg in CFA. Solid chow containing 2 % CVE was given to mice for 2 weeks before casein/CFA injection and was also given after injection throughout the experiments. Blood samples were obtained from the mice 3 weeks after casein/CFA injection, and the concentration of anti-casein IgE in the serum was determined by ELISA. The data are means±SD of 12 mice per group. *: $p< 0.05$, significantly different from untreated mice immunized with casein/CFA.

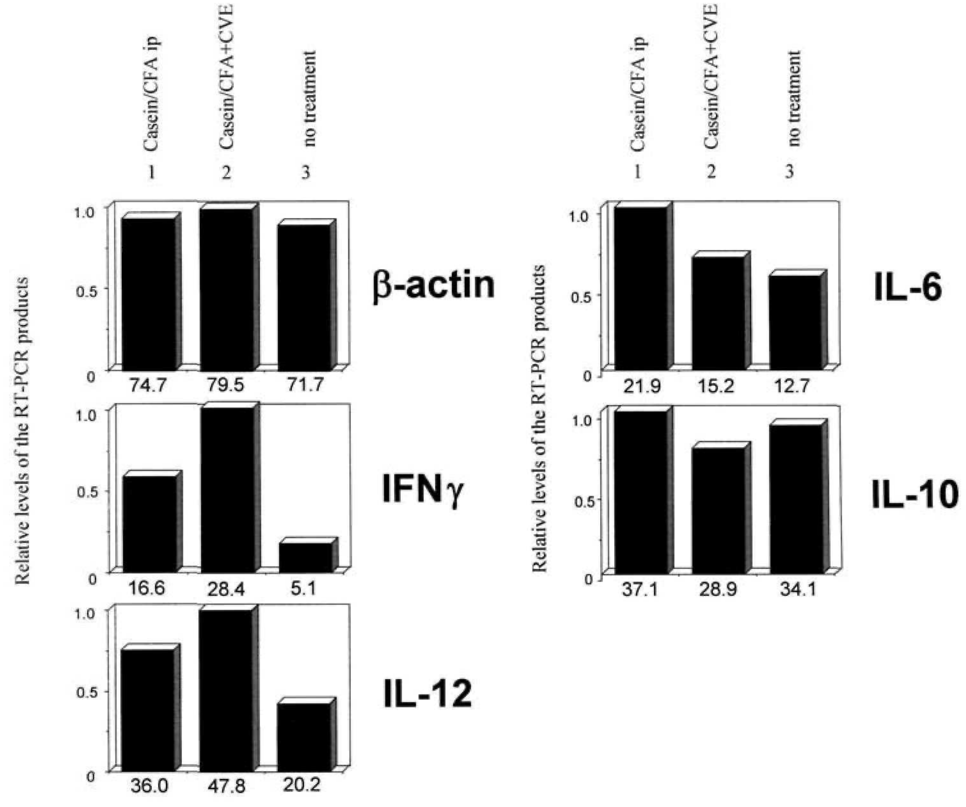


Figure 9 RT-PCR analysis of cytokine mRNA expression in the spleens of DBA/2 mice immunized with casein/CFA. Total RNA was extracted from pooled spleens of 5 DBA/2 mice or CVE-administered mice 3 weeks after immunized using RNAzol™ and reverse-transcribed, and the cDNA concentration was adjusted by co-amplification of two fold serially diluted cDNA and constant amounts of control plasmid *pMCQ* with β-actin specific primers. Cytokine expression patterns of IL-12, IFNγ, IL-6, and IL-10 were determined by PCR using murine cytokine-specific primers. The PCR products were separated in 2 % agarose gel and visualized by ethidium bromide staining. Relative quantification of the amounts of the RT-PCR products was performed using a Computing Densitometer and NIH Image™ software.

In order to confirm the effects of oral administration of CVE on the IL-12 and IFNγ mRNA expressions in the spleens of mice immunized with casein/CFA, we compared the expression of cytokine genes in the spleens of untreated and CVE-treated mice after immunization with casein/CFA. Total RNA was extracted from the spleens 3 weeks after immunization. The levels of IL-12 and IFNγ were augmented in the

spleens of CVE-treated mice. These results suggested that oral administration of CVE may stimulate IL-12 production and Th1 response.

Here, we showed that the production of casein-specific IgE Ab in mice immunized with casein/CFA was suppressed by oral administration of CVE, and that the production of casein-specific IgG2a levels was enhanced by oral administration of CVE, whereas the expressions of IL-12 and IFNγ mRNAs were increased in the spleens of mice after immunization with casein/CFA. These results suggested that CVE may be useful as a therapeutic or prophylactic agent to control allergy accompanied by the elevation of allergen-specific IgE Abs.

In conclusion, CVE may be beneficial for the prophylaxis of allergic diseases in which allergen-specific Th2 responses are predominant.

### Antitumor immuno-active glycoprotein from culture supernatant of *Chlorella vulgaris strain CK*

The growth of transplantable Meth A tumor was significantly suppressed in an Ag-specific manner when CVE was administered by not only an i.p. route [30], but orally as well [31]. It has been demonstrated that glycoprotein-rich substance (CVS) from the culture supernatant of *Chlorella vulgaris strain CK* displayed antitumor effect against experimental metastasis induced by Meth A fibrosarcoma [32].

We have recently identified an active substance (ARS-2) purified from CVS showing antitumor activity in a mouse model [33]. ARS-2 was a glycoprotein with a molecular weight of 63,100 amu, which consists of carbohydrate moiety having a β-1, 6-D-galactopyranose backbone and protein moiety.

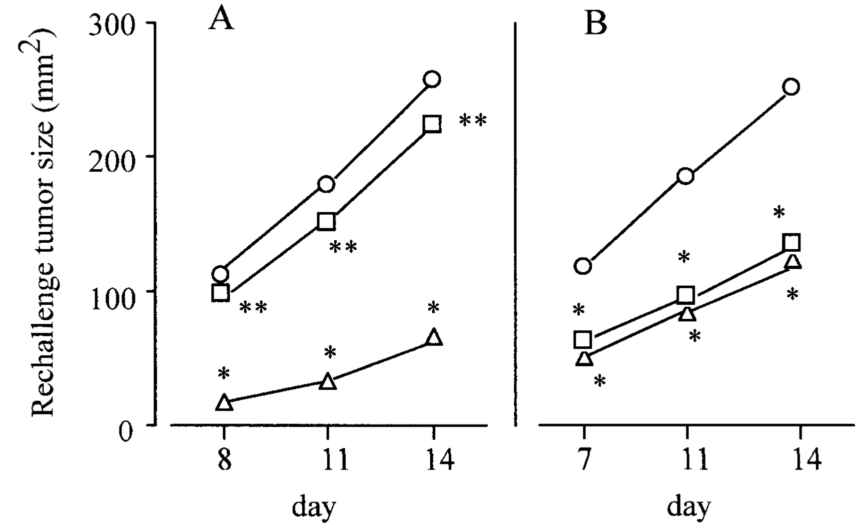


Figure 10 Influence of protease digestion (A) and controlled Smith degradation (B) on the antitumor activity of ARS-2. The sizes of rechallenged tumors are given after i.t. injection of protease (A, □) or inactivated protease(A, △) treated ARS-2, native ARS-2 (B, □), ARS-2 degraded by controlled Smith degradation (B, △), or PBS (○). *: Significantly different from the PBS group. ($p<0.05$). **: Significantly different from the inactivated protease-treated ARS-2 group. ($p<0.05$).

As shown Figure (**10**), the antitumor activity of ARS-2 was sensitive to proteolysis, but resistant to enzyme hydrolysis and periodate oxidation of the carbohydrate moiety, suggesting that the protein moiety is necessary for the antitumor activity of ARS-2 [33].

## III. Discovery of signal transduction systems via toll-like receptors (TLR) by ARS-2, an active substance showing antitumor activity from a glycoprotein-rich substance (CVS) from culture supernatant of *Chlorella vulgaris strain CK*

### What are toll-like receptors?

Toll was first identified as a protein that controls dorsoventral pattern formation in the early development of *Drosophila* [34] and was shown to participate in anti-microbial immune responses [35]. Recently, several mammalian Toll homologues have been identified and been proven to play important roles in the pattern recognition of various bacterial components. Among them, TLR2 and TLR4 have been shown to be involved in signaling from lipoproteins and lipid A, respectively.

Among them, TLR4 has been shown to recognize LPS from

Gram-negative bacteria [36], while TLR2 recognizes peptidoglycan and lipopeptide from Gram-positive bacteria and zymosan from yeast [37-38].

**TLR4 is not involved in the IL-12 p40 production by spleen-adherent cells stimulated with ARS-2**

IL-12 is a heterodimic cytokine composed of a constitutively expressed 35 kD (p35) subunit and an inducible 40 kD (p40) subunit. IL-12 has been shown to enhance the abilities of NK cells, Th1 cells, and cytotoxic T cells to produce IFNγ. IL-12 stimulation of NK cells and Th1 cells to produce IFNγ results in inhibition of Th2-type immune response [39-40].

In order to determine whether TLRs are involved in the recognition of antitumor glycoprotein, ARS-2, from the culture medium of *Chlorella vulgaris strain CK*, we examined IL-12 p40 production by the spleen-adherent cells of mice lacking functional TLR2 or TLR4 in response to ARS-2 in vitro [41].

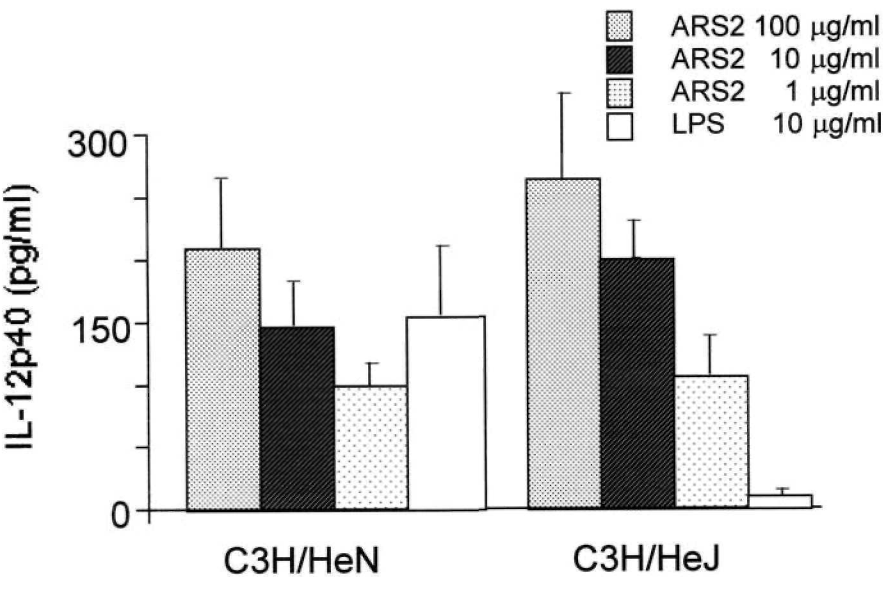


Figure 11 IL-12 production by adherent cells from the spleen of C3H/HeN or C3H/HeJ mice in the presence of ARS-2.

Adherent cells ($1x10^6$/well) from the spleen were incubated for 24 h in the presence of ARS-2 (1, 10, 100 μg/ml), and the culture supernatant was then collected. IL-12 p40 levels in the culture supernatant were determined by means of ELISA. The data are representative of three separate experiments and are expressed as the means of triplicates±SD.

As shown in Figure (**11**), the spleen-adherent cells produced rather higher

levels of IL-12 p40 in TLR4-deficient C3H/HeJ mice in response to stimulation with ARS-2 in a dose-dependent manner. These data imply that the cellular response to ARS-2 is not due to LPS contamination but is associated with the protein moiety of ARS-2.

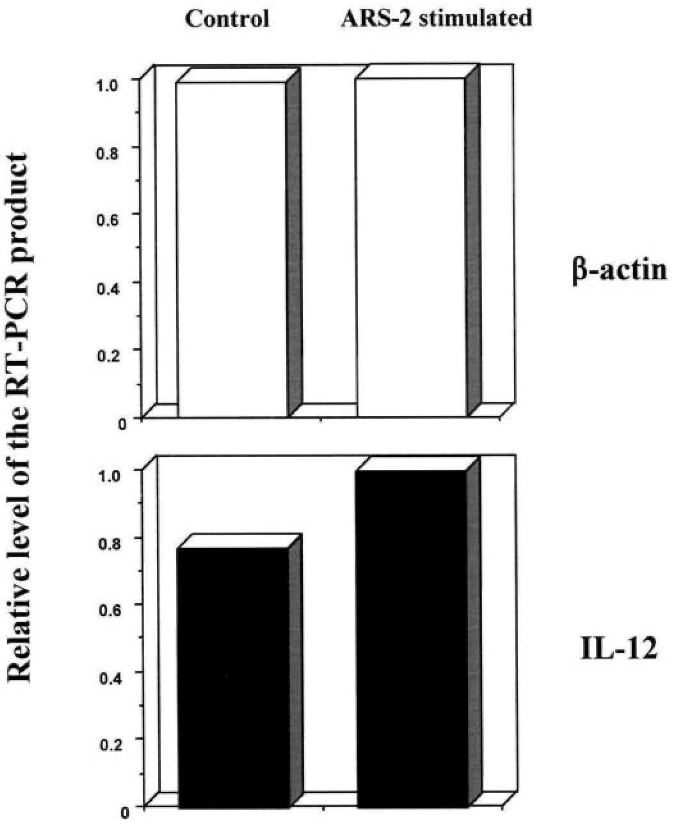


Figure 12 RT-PCR analysis of IL-12 mRNA expression in the spleen-adherent cells cultured with ARS-2. Total RNAs were extracted from the spleen-adherent cells cultured with ARS-2 (100 μg/ml) and reverse-transcribed. Cytokine mRNA expression patterns of β-actin and IL-12 were determined by PCR using murine cytokine-specific primers, respectively. The PCR products were separated in 2 % agarose gel and visualized by ethidium bromide staining. Relative quantification of the amounts of the RT-PCR products was performed using a Computing Densitometer and MasterScan™ software.

The spleen-adherent cells from C3H/HeJ mice were cultured for 48 h with ARS-2 (100 μg/ml), and IL-12 p40 levels in the spleen-adherent cells were determined by RT-PCR (Fig.(**12**)). Our results showed that the levels of IL-12 p40 mRNA was augmented in the spleen cultured with

ARS-2. Thus, TLR4 is not involved in ARS-2-mediated IL-12 p40 production by spleen-adherent cells.

**Impaired ability of TLR2-deficient (TLR2-/-) mice to produce IL-12 p40 in response to ARS-2**

Among TLRs, TLR4 has been shown to recognize LPS from gram-negative bacteria, while TLR2 recognizes peptidoglycan and lipopeptide from gram-positive bacteria. We next examined the production of IL-12 p40 in the supernatant of spleen-adherent cells co-cultured with ARS-2 in TLR2+/- and TLR2-/- mice.

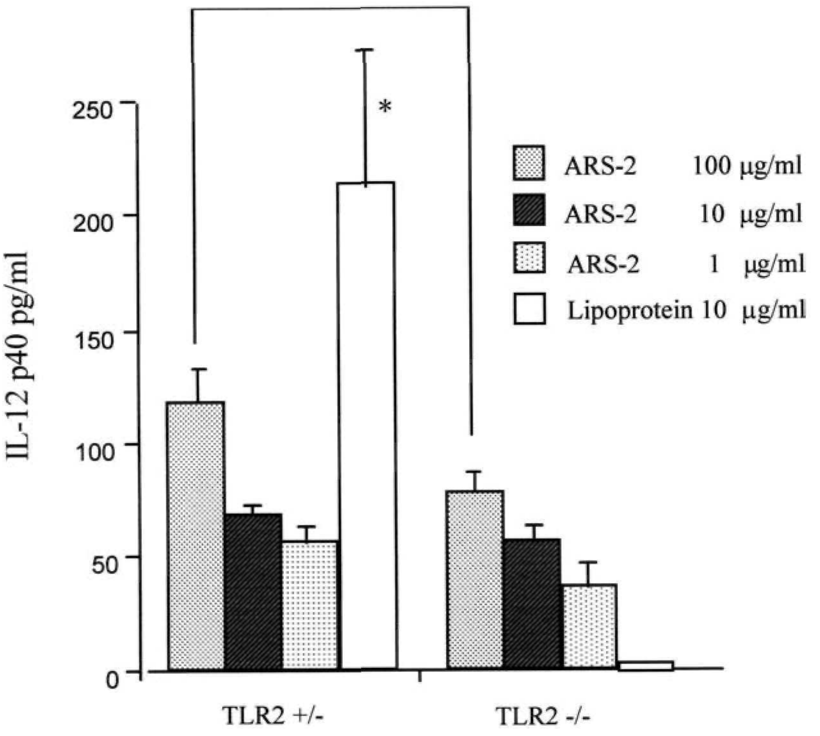


Figure 13 IL-12 production by adherent cells from the spleen of TLR2+/- and TLR2-/- mice in response to ARS-2. The adherent cells ($1 \times 10^6$/well) from the spleens of TLR2+/- and TLR2-/- mice were incubated for 24 h in the presence of ARS-2 (1, 10, or 100 μg/ml), and the culture supernatant was then collected. IL-12 p40 levels in the culture supernatant were determined by means of ELISA. The data are presented as means±SD of triplicate samples. *: $P<0.05$ vs the control group.

IL-12 production of the supernatant co-cultured with ARS-2 was increased in a dose-dependent manner in TLR2+/- mice, whereas IL-12

production was significantly impaired in the TLR2-/- mice. These results suggested that TLR2 may be at least partly involved in ARS-2 mediated cellular activation.

## Expression of mouse TLR2-conferred, ARS-2-mediated NF-kB activation in a human cell line

To confirm the involvement of TLR2 in ARS-2-mediated cellular activation, we inserted the coding region of mTLR2 cDNA into a mammalian expression plasmid. The flag-tagged mTLR2 was transiently expressed in a human HEK 293 cell line in combination with mouse CD14.

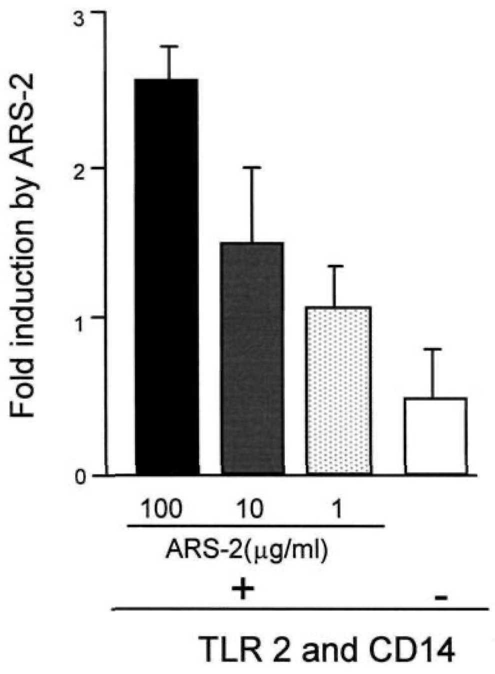


Figure 14 The biological responses of human HEK 293 cells to ARS-2 is reconstituted after transfection with murine CD14 and TLR2.

Human HEK 293 cells were plated onto 60-min plates at $1x10^6$ cell/plate on the day before transfection. Combinations of expression plasmid DNAs (TLR2 and CD14, each 6 μg total/plate) and 2 μg of pGL3-NF-kB/Luc (a luciferase reporter construct containing consensus NF-kB binding sequence) were transfected according to the manufacturer's instructions. Cells were harvested 48 h later and were stimulated with ARS-2 (1, 10, or 100 μg/ml) for 8 h and then lysed. Following this, luciferase activity was measured. Background luciferase activity was subtracted, and the data are presented as means±SD of triplicate samples.

The transfection of the control vector alone did not mediate the ARS-2-induced NF-kB activation, indicating that the parental HEK 293 cells were hyporesponsive to ARS-2. When TLR2 was coexpressed with CD14, ARS-2 significantly induced NF-kB activation (Fig.(**14**)).

Our present results showed that IL-12 and TNF-α production in response to ARS-2 was significantly reduced, but not completely abolished in the spleen-adherent cells from TLR2-deficient mice. These results indicate that ARS-2 activates the cells in a TLR2-dependent manner but not in a TLR4-dependent manner. We further confirmed that overexpression of mTLR2 and mouse CD14 conferred ARS-2-inducibility of nuclear factor NF–κB activation to human HEK 293 cells. TLR2 signaling is at least partly involved in the antitumor activity of the water-soluble antitumor glycoprotein from *Chlorella vulgaris strain CK.*

We show here several lines of evidence that TLR2 is required for cellular responses to ARS-2. Induction of IL-12 production by macrophages/dendritic cells via TLR2 signaling may be a major mechanism whereby *Chlorella vulgaris* promotes Th1–biased response to tumor and microbial infection. Further experiments with TLR2-deficient mice in vivo may clarify the potential role of TLR2 in various immunological effects of *Chlorella vulgaris strain CK.*

## III. Creation of new *Chlorella*-stored physiologically effective components

### III-1. γ-Aminobutyric acid (GABA)-rich *Chlorella*

#### Biosynthesis and accumulation of γ-Aminobutyric acid (GABA) in *Chlorella vulgaris strain CK*

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the vertebrate brain [42]. GABA is not confined to the central nervous system. GABA has been detected in the peripheral nervous system and in several nonneural tissues where it may play diverse physiologic roles [43]. Indeed, GABA has been reported to reduce blood pressure in experimental animals when it is administered systemically or orally, and it has been suggested that systemic administration of GABA induced the hypotensive effect associated with the blockade of sympathetic ganglia [44-46].

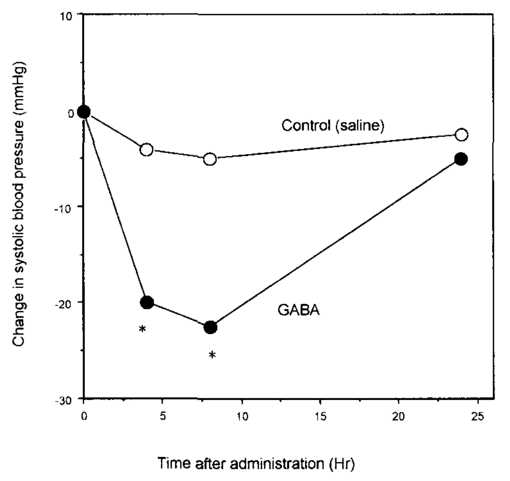


Figure 15 Effects of a single oral administration of GABA on systolic blood pressure in spontaneously hypertensive rats. Systolic blood pressure was measured by the tail-cuff method after keeping at 37°C for a few minutes in a constant temperature box. Each rat received either GABA (0.5 mg/kg, p.o.) dissolved in saline or saline alone. Each point represents the mean for 5-6 animals.

*: Significantly different from control ($P<0.01$).

Hayakawa et al. examined the effects of GABA on the blood pressure in spontaneously hypertensive rats (SHR). A single oral administration of GABA (0.5 mg/kg) significantly lowered the systolic blood pressure in SHR (Fig.(**15**)). The falls of systolic blood pressure were 20.8 mmHg at 4 h and 22.8 mmHg at 8 h after oral administration of GABA, respectively [46].

GABA is metabolized by three enzymes: glutamic acid decarboxylase (GAD), which produces GABA from glutamic acid, and the catabolic enzymes GABA-transaminase, and succinic semialdehyde dehydrogenase. A study of the metabolic reaction between γ-aminobutyric acid and glutamic acid was performed regarding *Chlorella cells* by Kating [47]. In *Chlorella* cells, glutamic acid is converted by GAD into GABA by direct decarboxylation. Therefore, we examined the manufacturing process of GABA-rich *Chlorella*, highly accumulated GABA, converted from glutamic acid by GAD in the cells.

At first, we examined the method for increasing the glutamic acid content in *Chlorella* cells. Reisner et al. reported that relative increases of glutamic acids were considerably greater when ammonium was provided as the nitrogen source [48]. *Chlorella vulgaris strain CK* accumulated 542 mg% of glutamic acid in the cells when ammonium was added to the medium after heterotrophic culture [49]. On the other hand, the contents of glutamic acid in the non-treated *Chlorella* cells was 158 mg%.

Secondly, we attempted to enrich the amounts of GABA in *Chlorella* cells. The glutamic acid-rich *Chlorella* cells produced 334 mg% of GABA in culture medium adjusted to pH 4.0 after 1 h-incubation at 38°C under anaerobic conditions.

**Effect of γ-Aminobutyric acid (GABA) on blood pressure in rats and humans.**

We examined the effects of GABA-rich *Chlorella* on blood pressure in SHR [49].

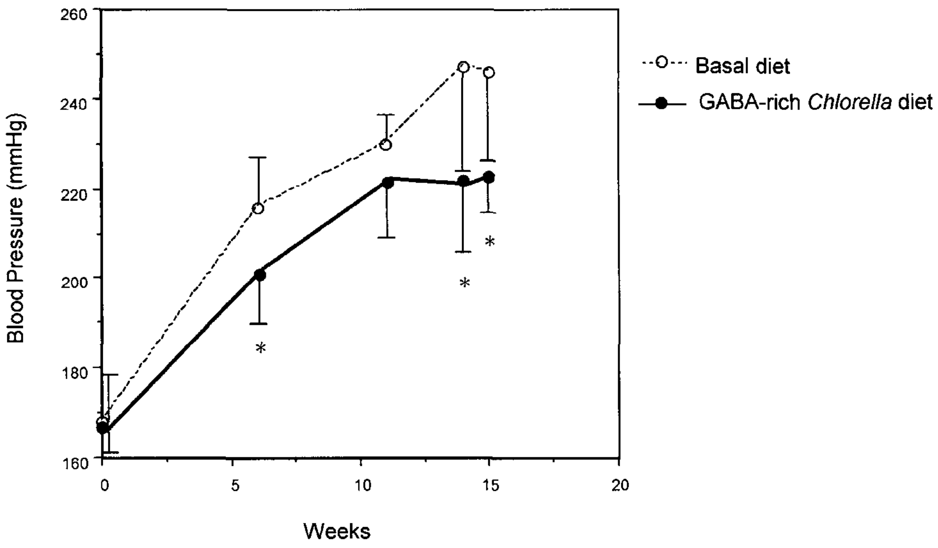


Figure 16 Effect of GABA-rich *Chlorella* on blood pressure in spontaneously hypertensive rats (SHR). The values are expressed as means±SD (n=6). Experimental diets containing 10 % GABA-rich *Chlorella* was given to SHR for 15 weeks and the measurement of blood pressure was executed using the tail-cuff method in all instances.

*: Significantly different from basal group ($p < 0.05$).

Elevation of blood pressure in SHR was significantly depressed by 22 mmHg in the GABA-rich *Chlorella* group compared with the basal group. The biochemical status showed that serum triglyceride (TG) and blood urea nitrogen (BUN) levels were significantly lower in SHR fed the

GABA-rich *Chlorella* diet compared with those fed the basal diet (data not shown).

Next, we examined the effects of GABA-rich *Chlorella* on blood pressure in mild hypertensive subjects [50]. Ten subjects (age, 48.8±3.5 years old; body weight, 73.4±7.4 kg; systolic blood pressure, 147.5±9.3 mmHg; diastolic blood pressure, 88.3±4.7 mmHg) took 6 g of GABA-rich *Chlorella* per day for 8 weeks.

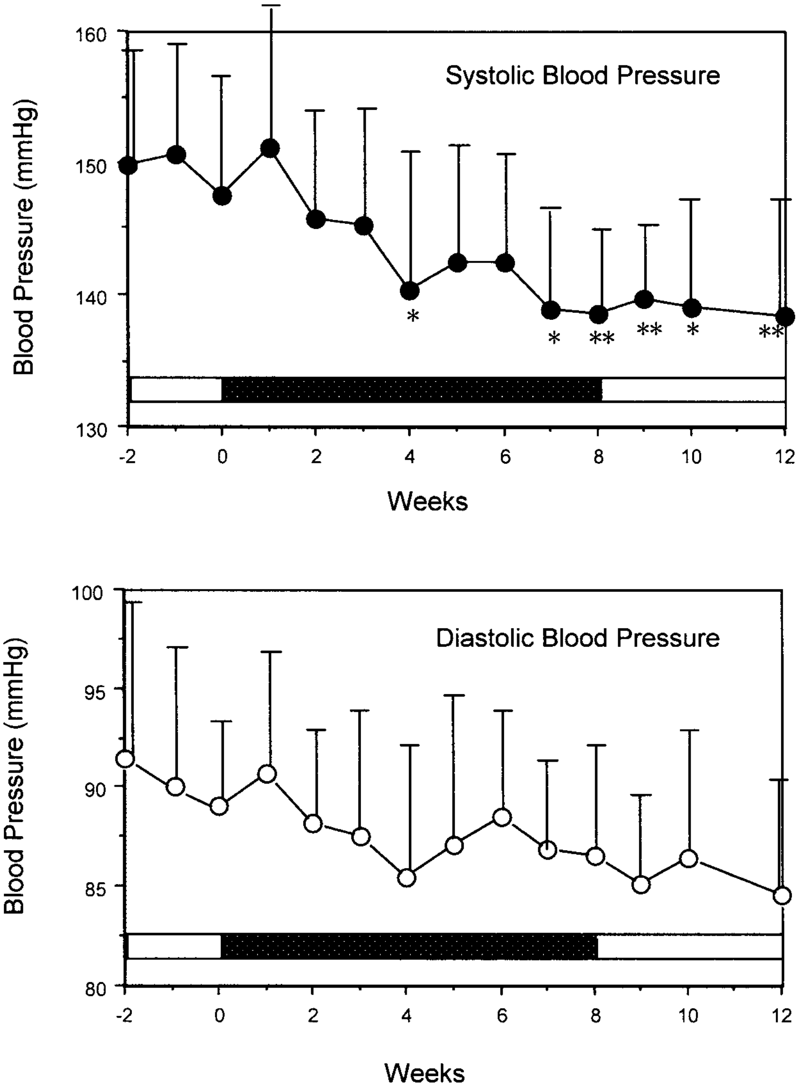


Figure 17 Effects of the GABA-rich *Chlorella* on blood pressure in the mild hypertensive subjects. Mild hypertension subjects (n=10) ingested 6 g of GABA-rich *Chlorella* per day and the blood pressure was measured once a week for 12 weeks.

Test-period ; observation-period

1. Values are means±SD, n=10.

2. Significantly different from the value at 0 week (*P<0.05; **P<0.01).

The systolic blood pressure was significantly lower (7.2 mmHg at 4 weeks; 9.0 mmHg at 8 weeks) after GABA-rich *Chlorella* intake as compared with the value before the intake. Diastolic blood pressure was unchanged after GABA-rich *Chlorella* intake. Neither body weight nor heart rate changed after GABA-rich *Chlorella* intake. Reductions in adrenaline, noradrenaline, and dopamine levels were observed to have been caused by GABA-rich *Chlorella* intake. The results suggested that GABA-rich *Chlorella* may have a hypotensive effect on mild hypertensive subjects through the suppression of the sympathetic nervous system.

Moreover, in order to clarify a hypotensive effect of GABA-rich *Chlorella*, a placebo-controlled clinical study was performed to evaluate the effect and effective dose of GABA-rich *Chlorella* in high-normal and mild hypertensive subjects [51]. Sixty adult participants (47.8±10.0 years old, mean±SD) with high-normal blood pressure or mild hypertension (systolic blood pressure: 145.3±5.9 mmHg, diastolic blood pressure: 87.7±6.5 mmHg) were randomly divided into 4 groups (15 participant per group). Participants ingested 2 g, 4 g, or 6 g of GABA-rich *Chlorella* per day, respectively, and participants in the placebo group ingested 4g of lactose, throughout the eight weeks treatment interval, followed by a two-week withdrawal observation.

Systolic blood pressures in the 4g or 6g GABA-rich *Chlorella* groups were significantly reduced at six and eight weeks after GABA-rich *Chlorella* administration in comparison with the values of the placebo group. There was also a tendency toward a decrease of diastolic pressure. Two weeks after discontinuation of GABA-rich *Chlorella* intake, the systolic blood pressure of the 4g or 6g GABA-rich *Chlorella* groups showed a tendency to rise, while no change was observed in the placebo group. No side effects were observed, and there were no remarkable clinical changes in the parameters of hematological, biochemical, or urine examination for a period of 0-8 weeks in any group. These results indicate that GABA-rich *Chlorella* improves the blood pressure in high-normal blood pressure and mild hypertensive subjects without side effects in subjective symptoms or clinical signs.

It has been reported that GABA was absorbed in the gastrointestinal tract as a specialized transport mechanism which obeys Michaelis-Menten and first-order kinetics [52]. Since the blood-brain barrier is impermeable to GABA [53], the hypotensive effect of peripherally administered GABA is due to the actions of the peripheral nervous system and those of several

nonneural tissues. It has also been suggestted that GABA has an hypotensive effect due to its inhibition of noradrenaline release from sympathetic nerves via presynaptic $GABA_B$ receptors in SHR[46]. When sympathetic nerve activity is high, GABA is able to reduce the activity via the inhibition of noradrenaline release. The GABA-induced hypotensive effect may be due to its inhibition of noradrenaline release from sympathetic nerve fibers.

Murakami et al. reported that *Chlorella* administration suppressed the rise of blood pressure and elongated the life-span in stroke-prone spontaneously hypertensive rats (SHRSP) [54]. The mechanism of hypotensive action and the elongation of the life-span by *Chlorella* administration involved the lowering of the plasma rennin activity, and depressing the reduction of elastin content in the aorta of SHRSP. Moreover, it has been reported that intravenous, intraperitoneal, or oral administration of *Chlorella* alkali extract showed a fall of the blood pressure in SHRSP [55]. Thus, *Chlorella* was expected to have a hypotensive effect in SHRSP.

The hypotensive components in *Chlorella* were estimated to be nucleic acids, and the digestive products of proteins and minerals such as potassium.

GABA-rich *Chlorella* contains GABA, which shows a hypotensive effect associated with the blockade of sympathetic ganglia and hypotensive components such as nucleic acids and proteins in *Chlorella* cells. Therefore, GABA-rich *Chlorella* is expected to show a synergistic effect on SHRSP or hypertensive subjects.

In conclusion, GABA-rich *Chlorella* seems to not only decrease high blood pressure but also prevent blood pressure elevation in humans and SHR. The administration of GABA-rich *Chlorella* might become one of the supportive methods to decrease blood pressure in essential hypertension. On the basis of these results, we propose that GABA-rich *Chlorella* might be beneficial for normalizing high blood pressure.

### III-2. Docosahexaenoic acid (DHA)-rich *Chlorella*

Several studies have shown the protective role of dietary (n-3) polyunsaturated fatty acids (PUFA) against cardiovascular diseases [56-57]. Greenland Eskimos and, to a lesser extent, some Japanese, with high dietary intake of (n-3) PUFA from seafood, have a low incidence of cardiovascular diseases compared with Western populations [58].

In fact, unlike saturated fatty acids and cholesterol, which have been implicated in atherogenesis, (n-3) PUFAs such as eicosapentaenoic acid and docosahexaenoic acid (DHA) are considered to be vasodilators and antihypertensive agents. DHA, a 22-carbon fatty acid with six double bonds, is one of the major n-3 highly PUFAs in fish oils, and it is highly enriched in mammalian brain phospholipids.

Recently, it was found that the intake of DHA effectively enhances maze-learning ability and brain functions in old mice rather than in young mice [59]. This study suggests that DHA is an important component for maintaining and improving brain functions in aged animals. Moreover, dietary supplement with DHA has provided evidence of its cardiovascular protective effects and its ability to lower blood pressure [60]. The mechanisms leading to these protective effects remain unclear.

Hayashi et al. demonstrated that DHA-rich *Chlorella* can be prepared by supplementing the culture media with purified DHA-enriched fish oil [61]. *Chlorella* was capable of incorporating exogenous free DHA from tuna oil-hydrolyzates into the cells; however, intact tuna oil was not incorporated into the cells. These results show that DHA-rich *Chlorella* can be easily prepared by supplementing the culture media with tuna oil-hydrolyzates.

In the DHA-rich *Chlorella* oil fraction, DHA is incorporated not only into neutral lipids, such as those in fish oils, but also into glycolipids and phospholipids [62]. The glycolipid of DHA-rich *Chlorella* consisted of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol. The phospholipid of DHA-rich *Chlorella* consisted of phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, and so on.

Recently, it was reported that these polar lipid-form DHAs showed more pharmacological effects than the neutral lipid-form [63]. Thus, this DHA-rich *Chlorella* may be anticipated as more efficient for supplementing DHA.

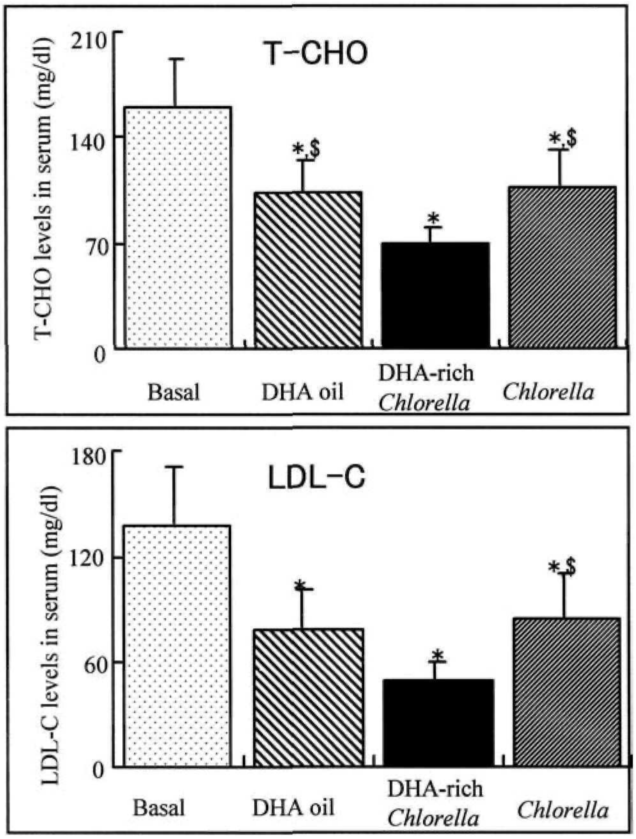


Figure 18 Anti-hypercholesterolemic effect of DHA-rich *Chlorella* in rats fed high cholesterol diet. DHA contents of DHA-rich *Chlorella*, DHA oil, and *Chlorella* diet are 0.8 %, 0.8 %, and 0 %, respectively. Values are menas±SD. *: Significantly different from the basal group ($P<0.05$); $: Significantly different from the DHA-rich *Chlorella* group ($P<0.05$).

The anti-hyperlipidemic effect of DHA-rich *Chlorella vulgaris* has been studied in a rat model [64]. The results demonstrated that the most promotive anti-hyperlipidemic action was found in the DHA-rich *Chlorella* group rather than in the DHA oil group or the *Chlorella* group. The anti-hyperlipidemic action of DHA-rich *Chlorella* may be synergistic effect between DHA oil and *Chlorella* components.

More recently, it was found that the intake of DHA effectively enhances maze-learning ability and brain functions in old mice rather than in young mice, as mentioned above [59]. Thus, DHA is an important component for maintaining and improving brain functions in aged animals. More recently, Sugimoto et al. examined the effect of DHA-rich *Chlorella* oil administration on maze learning ability [65].

The effects of DHA-rich *Chlorella* oil administration for six weeks on total errors, reference memory errors, and working memory errors regarding radial maze performance in mice were shown Fig.(**19**). The administration of DHA-rich *Chlorella* oil significantly decreased the numbers of total errors and working memory errors. These results showed that the intake of DHA-rich *Chlorella* oil effectively enhances working memory in maze performance.

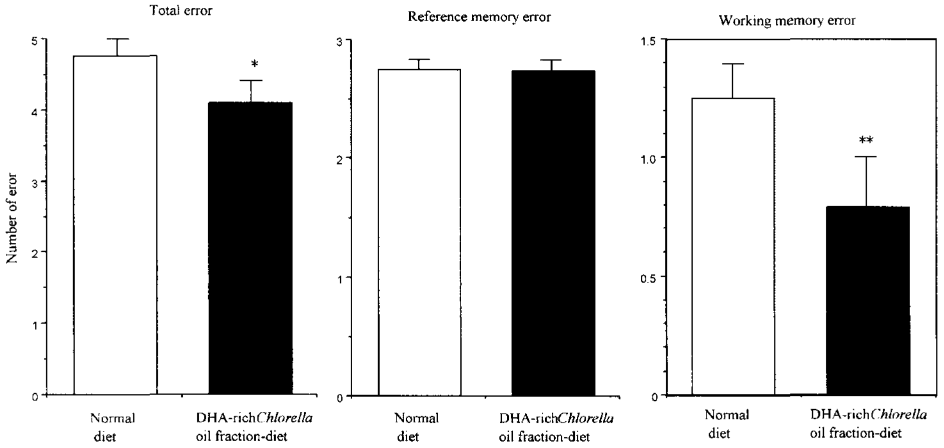


Figure 19 Effect of DHA-rich *Chlorella* oil-diet on eight-arm radial maze performance of aged mice. Each column and vertical bar represents the means±SE of 20 trials (5-7 mice).

*: Significantly different from the normal diet group ($P<0.05$).

**: Significantly different from the normal diet group ($P<0.01$).

*Chlorella* and a higher plant couldn't synthesize DHA or EPA. Therefore, DHA-rich *Chlorella* appears to be a promising candidate for the prevention of life style-related diseases which develop in the elderly subjects. The anti-hyperlipidemic effect was shown in the DHA-*Chlorella* group, and the elevation of total cholesterol levels in serum was suppressed

## Conclusions

1, *Chlorella* containing chlorophyll and dietary fiber seems to protect against health disorders caused by dioxin exposure by capturing dioxin in the digestive tract by diminishing the amount of dioxin absorption. Thus,

*Chlorella* is useful for promoting dioxin excretion.
2, Oral administration of hot water extracts of *Chlorella vulgaris strain CK* (CVE) augments the resistance against *Listeria* infection via development of cell-mediated immunity to *Listeria.* An increase in the number of γδ T cells at the early stage and that in the number of αβ T cell, especially $CD4^+$ T cells, at the late stage after infection was evident in CVE-administered mice. Early appearing γδ T cells may be important for the induction of subsequent cell-mediated immunity.
3, We demonstrated that oral administration of CVE caused the retention of the capacity of MAIDS mice to eliminate *L.monocytogenes* in association with an improvement of the defective immune response to *L.monocytogenes*. CVE is somewhat effective in the treatment of opportunistic *Listeria* infections in immuno-deficient mice.
4, We showed that the production of casein-specific IgE Ab in mice immunized with casein/CFA was suppressed by oral administration of CVE, whereas expressions of IL-12 and IFNγ. mRNAs was increased. Our results suggest that CVE may be beneficial in the prophylaxis of allergic diseases in which allergen-specific Th2 responses are predominant.
5, Induction of IL-12 produced by macrophages/dendric cells via TLR2 signaling may be a major mechanism whereby *Chlorella vulgaris strain CK* promotes Th1-biased response to tumor and microbial infection. Further experiments with TLR2-deficient mice in vivo may clarify the potential role of TLR2 in various immunological effects of *Chlorella vulgaris.*
6, Damages to blood vessels and target organs are triggered by hypertension itself. As an urgent problem in our aging society involves increasing numbers of hypertensive subjects, GABA-rich *Chlorella* would appear to be a candidate for the prevention of high blood pressure in hypertensive subjects.
7, DHA-rich *Chlorella* administration showed a synergistic effect on anti-hyperlipidemic action. The anti-hyperlipidemia effect of DHA-rich *Chlorella* administration may be stronger rather than DHA or *Chlorella* administration alone. Moreover, administration of DHA-rich *Chlorella* oil significantly decreased the numbers of total errors and working memory errors in mice. DHA-rich *Chlorella* can be easily prepared by supplying DHA to the culture medium. In the future, DHA-rich *Chlorella* would be expected to prevent adult disease such as hyperlipidemia, hypertention, and senile dementia.

## ABBREVIATIONS

| | | |
|---|---|---|
| Ab | = | Antibody |
| AIDS | = | Acquired immunodeficiency syndrome |
| amu | = | Atomic mass units |
| Bio | = | Biotin |

| | | |
|---|---|---|
| CD | = | Cluster of differentiation |
| cDNA | = | Complementary DNA |
| CFA | = | Complete Freund's adjuvant |
| CSF | = | Colony-stimulating factor |
| CTL | = | Cytotoxic T lymphocytes |
| CVE | = | Hot water extracts of *Chlorella vulgaris* |
| CVS | = | Glycoprotein-rich substance |
| DHA | = | Docosahexaenoic acid |
| DMBA | = | 7, 12-dimethylbenzantracene |
| DMH | = | 1, 2-dimethylhydrzine |
| DTH | = | Delayed-type hypersensitivity |
| ELISA | = | Enzyme-linked immunosorbent assay |
| FCM | = | Flow cytometry |
| FITC | = | Fluorescein isothiocyaniate |
| GABA | = | γ-aminobutyric acid |
| GAD | = | Glutamic acid decarboxylase |
| GM-CSF | = | Granulocyte-macrophage colony-stimulating factor |
| HEK 293 | = | Human embryonic kidney 293 |
| Ig | = | Immunoglobulin |
| IgE | = | Immunoglobulin E |
| i.p. | = | Intraperitoneal |
| IL-1α | = | Interleukin-1 alpha |
| IL-4 | = | Interleukin-4 |
| IL-5 | = | Interleukin-5 |
| IL-6 | = | Interleukin-6 |
| IL-10 | = | Interleukin-10 |
| IL-12 | = | Interleukin-12 |
| i.t. | = | Intratumoral |
| LPS | = | Lipopolysaccharide |
| mAbs | = | Monoclonal antibodies |
| MAIDS | = | Murine acquired immunodeficiency syndrome |
| Meth A | = | Murine methylcholanthrene induced fibrosarcomas |
| MIP | = | Macrophage inflammatory protein |
| mRNA | = | Messenger RNA |
| NF-κB | = | Nuclear factor κB |
| NK | = | Natural killer |
| $O_8$CDD | = | Octachlorodibenzo-*p*-dioxin |
| $O_8$CDF | = | Octachlorodibenzofuran |
| OD | = | Optical density |
| PBS | = | Phosphate buffered saline |
| PCB | = | Polychlorinated biphenyl |
| PCDD | = | Polychlorinated dibenzo-*p*-dioxin |

| | | |
|---|---|---|
| PCDF | = | Polychlorinated dibenzofuran |
| $P_5CDD$ | = | Pentachlorodibenzo-*p*-dioxin |
| $P_5CDF$ | = | Pentachlorodibenzofuran |
| PCR | = | Polymerase chain reaction |
| PE | = | R-phycoerythrin |
| PEC | = | Peritoneal exudates cell |
| PUFA | = | Polyunsaturated fatty acid |
| RT-PCR | = | Reverse transcription-polymerase chain reaction |
| SHR | = | spontaneously hypertensive rats |
| SHRSP | = | stroke-prone spontaneously hypertensive rats |
| $T_4CDD$ | = | Tetrachlorodibenzo-*p*-dioxin |
| $T_4CDF$ | = | Tetrachlorodibenzofuran |
| TCR | = | T cell receptor |
| TEQ | = | Toxic equivalents |
| TG | = | Triglyceride |
| Thl | = | T helper 1 |
| Th2 | = | T helper 2 |
| TLR | = | toll-like receptors |
| TNF$\alpha$ | = | Tumor necrosis factor alpha |

## References


[1] Sano, T.; Kumamoto, Y.; Okuda, M.; Tanaka, Y.; *XV International Congress of Nutrition*, **1993.**

[2] Singh, A.; Singh, S.P., Bamezai, R.; *Anticancer. Res*, **1999**, 19, 1887-1891.

[3] Morita, K.; Matsueda, T.; Iida, T.; Hasegawa, T.; *J. Nutr*, **1999**, 129, 1731-1736.

[4] Morita, K.; Ogata, M.; Hasegawa, T.; *Environ. Health. Perspect*, **2001**, 109, 289-294.

[5] Dashwood, R.H.; *Carcinogenesis*, **1992**, 13,113-118.

[6] Edenharder, R.; Leopoid, C.; Kries, M.; *Mutat.Res*, **1995**, 341, 303-318.

[7] Pore, R.S.; *Drug.Chem.Toxicol*, **1984,** 7, 57-71.

[8] Singh, A.; Singh, S.P.; Bamezai, R.; *Anticancer. Res*, **1998**, 18, 1509-1514.

[9] Singh, S.P.; Tiku, A.B.; Kesavan, P.C.; *Indian. J. Exp. Biol*, **1995,** 33, 612-615.

[10] Hasegawa, T.; Okuda, M.; Nomoto, K.; Yoshikai, Y.; *Immunopharmacol Immunotoxicol*, **1994**, 16, 191-202.

[11] Mitsuyama, M.; Takeya, K.; Nomoto, K.; Shimotori, S.; *J.Gen.Microbiol*, **1978,** 106:165-171.

[12] Kaufmann, S.H.; Simon, M.M.; Hahn, H.; *J.Exp.Med*, **1979,** 150-1033.

[13] Kaufmann, S.H.; *Immun. Today*, **1988**, 9, 168-174.

[14] Ohga, S.; Yoshikai, Y.; Takeda, Y.; Hiromatsu, K.; Nomoto, K.; *Eur.J.Immun*, **1990,** 20, 533-538.

[15] Hiromatsu, K.; Yoshikai, Y.; Matsuzaki, G.; Ohga, S.; Muramori, K.; Matsumoto, K.; Bluestone, J.A.; Nomoto, K.; *J.Exp.Med*, **1992**, 175, 49-56.

[16] Hasegawa, T.; Tanaka, T.; Yoshikai, Y.; *Int.Immunol*, **1992**, 4, 1129-1136.
[17] Konishi, F.; Tanaka, K.; Kumamoto, S.; Hasegawa, T.; Okuda, M.; Yano, I.; Yoshikai, Y.; Nomoto, K.; *Cancer.Immunol.Immunother*, **1990**, 32 , 1-7.
[18] Laterjet, R.; Duplan, J.F.; *Int.J.Radiat.Biol*, **1962**, 5, 339-344.
[19] Gazzinelli, R.T.; Makino, M.; Chattopadhyay, S.K.; Snapper, C.M.; Sher, A.; Hügin, A.W.; Morse III, H.C.; *J.Immunol*, **1992,** 148,182-188.
[20] Hügin, A.W.; Cerny, A.; Morse III, H.C.; *Cell.Immunol*, **1994**, 155, 246-252.
[21] Sher, A.;.Gazzinelli, R.T.; Oswald, I.P.; Clerici, M.; Kullberg, M.; Pearce, E.J.; Berzofsky, J.A.; Mossmann, T.R.; James, S.L.; Morse III, H.C.; Shearer, G.M.; *Immuno.Rev*, **1992,** 127, 183-204.
[22] Hasegawa, T.; Okuda, M.; Makino, M.; Hiromatsu, K.; Nomoto, K.; Yoshikai,Y.; *Int.J.Immunopharmac*, **1995**, 17, 505-512.
[23] Male, D.; Champion, B.; Cooke, A.; Owen, M.; *Advanced immunology Second Edition*, **1992**, chap.10.2 (Gower medical publishing, London/New York)
[24] Hsieh, C.S.; Macatonia, S.E.; Tripp, C.S.; Wolf, S.; O'Garra, A.; Murphy, K.M.; *Science*, **1993**, 260, 547-549.
[25] De Vries, J.E.; Gauchat, J.F.; Aversa, G.G.; Punnonen, J.; Gascan, H.; Yssel, H.; *Curr.Opin.Immunol*, **1991**, 3, 851-858.
[26] Umetsu, D.T.; Dekruyff, R.H.; *Proc. Soc.Exp.Biol.Med*, **1997**, 215, 11-20.
[27] Mossman, T.R.; Coffman, R.L.; *Annu.Rev.Immunol*, **1989,** 7, 145-173.
[28] Finkelman, F.D.; Holmes, J.; Katona, I.M.; Urban Jr, J.F.; Beckmann, M.P.; Park, L.S.; Schooley, K.A.; Coffman, R.L.; Mossmann, T.R.; Paul, W.E.; *Annu.Rev.Immunol*, **1990**, 8, 3030-3333.
[29] Hasegawa, T.; Ito, K.; Ueno, S.; Kumamoto, S.; Ando, Y.; Yamada, A.; Nomoto, K.; Yoshikai, Y.; Int. J. Immunopharmac, **1999,** 21, 311-323.
[30] Konishi, F.; Tanaka, K.; Himeno, K.; Taniguchi, K.; Nomoto, K.; *Cancer. Immunol. Immunother*, **1985**, 19, 73-78.
[31] Tanaka, K.; Tomita, Y.; Tsuruta, M.; Konishi, F.; Okuda, M.; Himeno, K.; Nomoto, K.; *Immunopharmac.Immunotoxicol*, **1990**, 12, 277-291.
[32] Tanaka, K.; Yamada, A.; Noda, K.; Hasegawa, T.; Okuda, M.; Shoyama, Y.; Nomoto, K.; *Cancer.Immunol.Immunother*, **1998,** 45, 313-320.
[33] Noda, K.; Ohno, N.; Tanaka, K.; Kamiya, N.; Okuda, M.; Yadomae, T.; Nomoto, K.; Shoyama, Y.; *Planta.Med*, **1996**, 62, 423-426.
[34] Belvin, M.P.; Anderson, K.V.; *Ann.Rev.Cell.Dev.Biol*, **1996**, 12, 393-416.
[35] Lemaitre, B.; Nicolas, E.; Michaut, L.; Reichhart, J.M.; Hoffmann, J.A.; *Cell*, **1996**, 86, 973-983.
[36] Takeuchi, O.; Hoshino, K.; Kawai, T.; Sanjo, H.; Takada, H.; Ogawa, T.; Takeda, K.; Akira, S.; *Immunity*, **1999**, 11, 443-451.
[37] Takeuchi, O.; Akira, S.; *Int.Immunopharmacol*, **2001**, 1, 625-635.
[38] Sankaran, K.; Wu, H.C.; *J.Biol.Chem*, **1994,** 269, 19701-19706.
[39] Bancroft, G.J.; Schreiber, R.D.; Unanue, E.R.; *Immunol.Rev*, **1991**, 124, 5-24.
[40] Hsieh, C.S.; Macatonia, S.E.; Tripp, C.S.; Wolf, S.F.; O'Garra, A.; Murphy, K.M.; *Science*, **1993**, 260, 547-549.
[41] Hasegawa, T.; Matsuguchi, T.; Noda, K.; Tanaka, K.; Kumamoto, S.; Shoyama, Y.; Yoshikai, Y.; *Int.Immunopharmacol*, **2002**, 2, 579-89

[42] Kish, P.E.; Fisher-Bovenkerk, C.; Ueda, T.; *Proc.Natl.Acad.Sci,* **1989**, 86, 3877-3881.

[43] Tillakaratne, N.J.; Medina-Kauwe, L.; Gibson, K.M.; *Comp.Biochem.Physiol,* **1995**, 112A, 247-263.

[44] Stanton, H.C.; *Arch.int.Pharmacodyn,* **1963,** 143, 195-204.

[45] Vemulapalli, S.; Barletta, M.; *Arch.int.Pharmacodyn,* **1984**, 267, 46-58.

[46] Hayakawa, K.; Kimura, M.; Kamata, K.; *Eur.J.Pharmacol,* **2002**, 438, 107-113.

[47] Kating, H.; *Planta,* **1958**, 51, 635-644.

[48] Reisner, G.S.; Gering, R.K.; Thompson, J.F.; *Plant.Physiol,* **1960**, 35, 48-52.

[49] Nakamura, T.; Matsubayashi, T.; Kamachi, K.; Hasegawa, T.; Ando, Y.; Omori, M.; *Nippon.Nogeikagaku.Kaishi,* **2000**, 74, 907-909.

[50] Nakamura, T.; Hasegawa, T.; Ueno, S.; Kumamoto, S.; Matsubayashi, T.; Ando,Y.; Kitsuki, K.; Omori, M.; Kitsuki, T.; *Jpn.Pharmacol.Ther,* **2000,** 28, 529-533.

[51] Tsuchida, T.; Mashiko, K.; Yamada, K.; Hiratsuka, H.; Shimada, T.; Itagaki, Y.; Fujinuma, H.; Samejima, K.; Nakamura, T.; Hasegawa, T.; Matsubayashi, T.; *J.Jpn.Soc.Nutr.Food.Sci,* **2003**, 56, 97-101.

[52] Nacher, A.; Polache, A.; Moll-Navarro, M.J.; Pla-Delfina, J.M.; Merino, M.; *Biopharm.Drug.Dispos,* **1994,** 15, 359-71.

[53] Kuriyama, K.,; Sze, P.Y.; *Neuropharmacology,* **1971**, 10, 103-108.

[54] Murakami, T.; Okamoto, K.; Ogaki, M.; Iizuka, Y.; *J.Jpn.Soc.Nutr.Food.Sci,* **1987**, 40, 351-359.

[55] Okamoto, K.; Iizuka, Y.; Murakami, T.; Miyake, H.; Suzuki, T.; *The congress of spontaneously hypertensive rats (SHR),* **1978**.

[56] Bonaa, K.H.; Bjerve, K.S.; Straume, B.; Gram, I.T.; Thelle, D.; *N.Eng.J.Med,* **1990**, 322,795-801.

[57] Stampfer, M.J.; Hu, F.B.; Manson, J.E.; Rimm, E.B.; Willet, W.C.; N.Eng.J.Med, **2000**, 343, 16-22.

[58] Robertson, T.L.; Kato, H.; Gordon, T.; Kagan, A.; Rhoads, G.G.; Land, C.E.; Worth, R.M.; Belsky, J.L.; Dock, D.S.; Miyanashi, M.; Kawamoto, S.; *Am.J.Cardiol,* **1998**, 39, 244-250.

[59] Lim, S.Y.; Suzuki, H.; *J. Nutr,* **2000**, 130, 1629-1632.

[60] Mori, T.A.; Bao, D.Q.; Burke, V.; Puddey, I.B.; Beilin, L.J.; *Hypertension,* **1999**, 34,253-260.

[61] Hayashi, M.; Yukino, T.; Maruyama, I.; Kido, S.; Kitaoka, S.; *Biosci. Biotecnol.Biochem,* **2001,** 65, 202-204.

[62] Yukino, T.; Tanaka, K.; Maruyama, I.; Konishi, F.; Kumagai, T.; Hada, T.; Hayashi, M.; *J.Jpn.Soc.Nutr.Food.Sci*, **2002**, 55, 331-337.

[63] Nojima, M.; Hosokawa, M.; Takahashi, K.; Hatano, M.; Fisheries. Sciences, 1994, 60, 729-34.

[64] Konishi, F.; Tanaka, K.; Maruyama, I.; Yukino, T.; Kumagai, T.; Hada, T.; Hayashi, M.; *J.Jpn.Soc.Nutr.Food.Sci,* **2002**, 55, 215-222.

[65] Sugimoto, Y.; Taga, C.; Nishiga, M.; Fujiwara, M.; Konishi, F.; Tanaka, K.; Kamei, C.; *Biol.Pharm.Bull,* **2002**, 25, 1090-1092.

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# PHARMACOLOGICAL ACTIVITY OF NEW μ, δ, κ RECEPTOR AGONISTS AND ANTAGONISTS

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**ABSTRACT:** Endogenous opioids have been studied extensively since their discovery, in the hope of findings a perfect analgesic, devoid of the secondary effects of alkaloid opioids. However, the design of selective opioid agonists and or antagonists has proved very difficult. First, structural studies of peptides in general are hampered by their intrinsic flexibility. Second, the relationship between constitution and the socalled "bioactive conformations" is far from obvious. Ideally, a direct structural study of the complex between a peptide and its receptor should answer both questions, but such a study is not possible, because opioids receptors are large membrane proteins, difficult to study by standard structural techniques. Thus, conformational studies of opioid peptides are still important for drug design and also for indirect receptor mapping.
This review deals the pharmacological activity of :

a) **a new μ and δ agonist**: The single amino acid replacement of 2',6'-dimethyl-L-tyrosine in deltorphin B (H-Dmt-D-Ala-Phe-Glu-Val-Val-Gly-NH2) yielded high affinity for mu- and delta-binding sites. [Dmt1]Deltorphin B lacks activity at kappa-opioid binding sites. Bioactivity *in vitro* with guinea-pig ileum confirmed that [Dmt1]deltorphin B interacted with mu-opioid receptors by reducing electrically induced contractions in a naloxone-reversible manner and was 150-fold more potent than morphine and comparable to [D-Ala2,NMePhe4,Gly-ol5]enkephalin (DAGO). The inhibition of spontaneous contractions of rabbit jejunum provided evidence for delta-opioid receptor interaction. Analgesia (hot plate and tail flick tests) revealed that [Dmt1]deltorphin B was 180- to 200-fold more potent than morphine. Pretreatment with naloxone, naltrindole or H-Dmt-Tic-Ala-OH (a highly selective delta-opioid receptor antagonist) prevented [Dmt1]deltorphin B antinociception. Thus, [$Dmt^1$]deltorphin B exhibited remarkably high dual affinity and bioactivity toward delta- and mu-opioid receptors.

b) **two new δ- and κ-opioid antagonist**: Substitution of 1,2,3,4 tetrahyidroisoquinoline-3-carboxylic acid (Tic) in place of Gly2 in dynorphin A-(1-13)-NH2 and A-(1-11)-NH2 (DYN) analogues (**1** and **2**) decreased the affinity to the kappa, delta, and mu receptors, and kappa selectivity. The analogue [D-Ala2, des-Gly3]DYN (**4**), a chimera between deltorphin/dermorphin N-terminal tripeptide and DYN, was virtually inactive for kappa-sites while the affinities for delta- and mu-receptors

remained essentially unchanged. The doubly substituted analogue [2',6'-dimethyl-L-tyrosine (Dmt1)-Tic2]DYN (**3**) exhibited high delta-affinity (Ki=0.39 nM) while mu- and kappa-affinities were only an order of magnitude less (4-5 nM). Bioactivity of [Tic2]DYN peptides (**1-3**) on guinea-pig ileum and rabbit jejunum revealed potent delta- and kappa-antagonism, while the delta agonist potency of 4 was comparable to DYN. Thus, conversion from a kappa-agonist to antagonist occurred with the inclusion of Tic into DYN analogues, similar to the appearance of antagonist properties with delta- and mu-opioid agonists containing a Tic2-residue.

c) **two new δ opioid peptide receptor antagonists (Dmt-Tic-OH (DTOH) and Dmt-Tic-Ala-OH (DTAOH)**: Dmt-Tic-OH (DTOH) and Dmt-Tic-Ala-OH (DTAOH), effective antagonists in vitro, represent a new potent opioid dipeptides for the delta-opioid receptor (Ki delta of 0.022 nM and a selectivity, Ki mu/Ki delta, of 150,000 for DTOH; Ki delta of 0.285 nM and a selectivity Ki mu/Ki delta, of 20,4 for DTAOH). In the present study we considered the pharmacological activity of these two new delta opioid peptide receptor antagonists in vivo. Therefore, we have evaluated their possible antagonistic activity against the antinociception induced by the highly selective delta opioid receptor agonist, [D-Ala2]deltorphin II (DEL). Furthermore, these two delta opioid peptide receptor antagonists were injected centrally or peripherally in order to assess their ability to act also after systemic administration. Concurrent i.c.v. injection of DTOH or DTAOH (0.5-1.0-2.0 nM) with DEL (5 nmol) induced a significant reduction of DEL antinociception. By contrast, while DTOH (10-20-40 mg/kg) administered peripherally (i.p., s.c. or i.v.) was also able to reduce DEL antinociception, DTAOH failed. The present results indicate that DTOH is the first opioid dipeptide with delta antagonist activity after systemic administration and it could be important in the clinical and therapeutic applications.

d) **a new μ selective opioid dipeptide antagonists**: the potent delta selective opioid antagonist dipeptides were designed on the basis of a simple conformational analysis. Following a similar procedure we found a mu selective dipeptide antagonist, 2,6-dimethyl-Tyr-D-Phe-NH2. Although its selectivity is not as high as those of the quoted delta selective dipeptides it has good *in vitro* activity and looks very promising for further development since the 2,6-dimethyl-Tyr-D-Phe message, like the delta selective 2,6-dimethyl-Tyr-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid counterpart, seems able to impart antagonism to longer peptides.

e) **deltorphin C and dermorphin with a D-glucopyranosyl moiety:** Deltorphin (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH2, DEL C) and dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2, DER) analogues in which a D-glucopyranosyl moiety is beta-O-glycosidically linked to a Thr4 or Thr7 side chain. Their activities were determined in binding studies based on displacement of mu- and delta-receptor selective radiolabels from rat brain membrane synaptosomes, in guinea pig ileum and rabbit jejenum bioassays, and, *in vivo*, by a mouse tail-flick test after intracerebroventricular (icv) and subcutaneous (sc) administrations. The glyco analogues modified at position 4 displayed low opioid properties, while Thr7-glycosylated peptides retained high delta- or mu-selectivity and remarkable activity *in vivo*. In particular, as systemic antinociceptive

agents, the latter glucoside-bearing compounds were more potent than the parent unglycosylated peptide counterparts, showing a high blood to brain rate of influx which may be due to the glucose transporter GLUT-1.

## INTRODUCTION

The opioid system consists of three main receptor types (μ, δ, κ) and their corresponding ligands (enkephalins, endorphins, dynorphins) which govern several physiological functions in peripheral tissues and central nervous system (CNS). [1-4] Recently a number of natural opioid peptides have been discovered endowed with significant receptor specificity: in particular endomorphins that have been found highly selective mu-receptor ligands [5] and two major classes of opioid peptides secreted from the skin of *Phyllomedusa* amphibians [6]. These heptapeptides,[6,7] called dermorphins (dermal morphine-like substances) DER, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-$NH_2$ [8] and deltorphins [9] (delta-specific opioids), DEL C, H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-$NH_2$ are potent and selective μ and δ agonists, respectively. μ, δ and κ receptors are inordinately sensitive to morphine and a veriety of alkaloid-derived agonists and antagonists [10]. They are traditionally applied extensively for the treatment of chronic [11,12] and acute [13] pain, as well as in the current therapy regimes to combat alcohol dependency [14] during immunosuppression in organ transplants [15] and in mediating the effects of narcotic addiction [16]. Alcaloid analgesic are very powerful and difficult to substitute with other drugs, especially for acute pain, but are plagued by several undesirable side effects: respiratory depression, gastrointestinal depression, and the development of tolerance and addiction.

All attempts to find the perfect analgesic, that is, an alkaloid retaining analgesic power comparable to that of morphine, but devoid of its secondary effects, failed. The discovery that all opioids act in the framework of a complex neurotrasmitter system that comprises at least three opioid receptors, that in principle might correspond to different actions, rekindled the hope of finding a powerful analgesic devoid of side effects. In effect many evidences indicate that analgesia mediated by agonist activation of delta opioid receptors [17] produces less marked side effects than analgesia mediated by mu-opioid receptors [18]. Consistently, since the discovery of natural selective opioid substances starting from these analogues, many efforts were aimed to the synthesis of compounds retaining opioid activity but improved farmacodinamic and farmacocinetic properties.

The basic step in the finding of the perfect analgesic is the knowledge of the bioactive conformation as a starting point for the synthesis of a rigid and powerful analog devoid of side effects.

The bioactive conformation is consistent with the farmacoforic points that are identified through structure activity relationship studies (SAR). SAR studies include the synthesis of many peptide and/or non peptide analogues displaying selectivity for one or another of the receptors and their pharmacologically defined subtypes [19] such as delta1- and delta2-opioid receprtor subtypes [20].

Due to their high selectivity, DER and DEL were the basis for an extensive structure activity relationship study aimed to understand the essential requisites of μ and δ activity. More than 200 analogues of DER and DEL C were synthesized, and the role of each amino acid in binding to μ and δ receptors and bioactivity was defined [21-31]. New synthetic products were prepared such as oxymorphindole and related derivatives [32], and (+)-4-[(alfa R)-alfa-((2S,5R)-4-allyl-2.5-dimethyl-l-piperazin-yl)-3-methoxy-benzyl]-N, diethylbenzamide [33]. These products are under investigation for their clinical relevance and provide potential non-peptide ligands for studies on delta-opioid-receptor.

As briefly reported the synthesis of an ideal analgesic asks for an improvement of both pharmacodynamic and in pharmacokinetic peculiarities. Sometimes if pharmacodynamic properties are improved, the pharmacokinetic characters can be still far from those of a perfect analgesic molecule. The quantities of opioids normally transported into the CNS can be low, and inadequate blood-brain barrier (BBB) permeation can be responsible for not optimized or for the different analgesic potencies among different opioid analogues. Consistently one major avenue of investigation involves the development of opioid analogues that penetrate the blood-brain-barrier (BBB).

DER and DEL as well as their synthetic analogues are generally resistant to enzymatic degradation, [34-35] and showed potent antinociceptive effects following intracerebroventricular administration, but only moderate or negligible central actions after systemic administration in mice and rats [36-38].

Alteration of the physiochemical properties of opioids, such as potentiating lipophilicity [39-42] or glycosylation in order to take advantage of the glucose transporter [43] represented efforts to enhance passage through the BBB. Following the latter strategy, Polt et al.[42] designed and prepared glycopeptide enkephalin analogues able to cross the BBB and to produce prolonged analgesia in mice [42]. These considerations have prompted to prepare and test DER and DEL C analogues in which D-glucopyranosyl units are beta-O-glycosidically linked to $Thr^4$ or $Thr^7$ side chains.

## Effects Of Dmt[1] In Deltorphin B.

Current evidence indicates that analgesia mediated by agonist activation of δ-opioid receptors [44] produces less marked side-effects than analgesia mediated by μ-opioid receptors [18]. Deltorphins which represent the potent family of opioid peptides originally isolated from frog skin [45] are the most selective δ-opioid receptor peptides. Thus,. they have served as parent peptide in the synthesis of numerous δ active peptide analogues.

Structural investigations had a basic role in driving the synthesis of new peptide and/or not peptide analogues. Essential indications on kinds and positions of new chemical moieties were given. Consistently it was found that the insertion of the 2',6'-dimethyl L-tyrosine (DMT) in the selective δ-opioid di and tri- peptides receptor antagonists [43, 46] confers a conformation to the peptide similar to that of its bioactive conformer [47]. On this basis deltorphin B was modified replacing Tyr with DMT and its activity was determined in pharmacological bioassay and radioligand binding assays for μ and δ opioid receptor.

## Bioassays.

**Transmurally stimulated guinea pig ileum (GPI).** Guinea pigs were killed with $CO_2$ and bled. Sections of GPI were preparated as follows. Segments of ileum 2-3 cm long were placed in a 10 ml organ bath containing Tyrode's solution with 5% $CO_2$ in 95% oxygen maintained at 37°C. The ileum preparation was placed between platinum electrodes and connected to a 85/5/20 MARB stimulator. A force displacement transducer and unirecord model polygraph were used for measurement of isotonic contraction. A resting tension of 0,5 g was applied and after 30 min equilibration period, the preparation was stimulated with a 0,5 ms pulse delivered transmurally at a frequency of 10 s at supramaximal voltage (25 V). Under these condictons, the preparation allows a contraction mean of 60 mm+/- 0.57. The inhibition of ileal contractions by drugs was expressed as percentage of basal value (means +/- S.E.M). Each analog was tested for its ability to inhibit electrically evoked contractions (i.e. tested for agonist activity) and to antagonize the inhibitory effects of μ agonists (DAGO, morphine, dermorphin). $PA_2$ values were calculated according to the procedure reported by Tallarida and Murray.

**Rabbit jejunum (RJ) preparation.** This is a new *in vitro* model to study δ opioid actiyity that we prefer to the customary MVD assay since the

jejunum contains mainly δ receptors whereas MVD also contains μ and κ receptors. The animals were killed with $CO_2$ and bled.

The abdomen was opened with a midline incision and three or four segments of jejunum (3 cm long) were removed from the same animal and placed in 10 ml tissue baths containing Tyrode's solution. The tissues were connected to an isotonic transducer by 1 g loading and allowed to equilibrate for 45 min; during this period regular spontaneous activity was recorded. Under these conditions, the preparation showed a contraction mean of 60 mm+/- 0.57, and the inhibition of rabbit contractions evaluated by the standard δ agonist (D-$Ala_2$) deltorphin II.

## Antinociception

To determine the nociceptive threshold of the mice, the hot plate test and the tail flick test were used. Drugs used are dissoved in sterile distilled water from icv administration immediately prior to injection at 5 μl/kg or 5μl mouse, respectively. The icv injection was performed and the site of administration of the peptide was verified in all animals by the injection of 1% methylene blue and the examination of the dye distribution in the cerebral ventricles at the termination of the experiment. To evaluate the hot plate and tail flick test responses detailed below, a control latency ($T_0$) was obtained from the mean of two latencies determined prior to drug injection; test latencies ($T_1$) were determined at various times after injection for each animal. The percentage of analgesia was calculated as ($T_1$ -$T_0$)/ ($T_2$ -$T_0$) x 100 where the cut off times ($T_2$) for the hot plate and tail flick test were 60 and 15s, respectively. The median antinociceptive dose (ED50) and 95% confidence limits were calculated according to the method of Litchfield and Wilcox.

## Bioactivity:

The high μ-opioid receptor affinity of peptide **1** was confirmed in the guinea-pig ileum bioassay *in vitro*. These experiments indicated that peptide **1** reduced the electrically-induced contractions of guinea pig-ileum while peptides **2** and **3** failed to cause significant inhibition up to μM concentrations (Table 1). Since naloxone at a concentration of 0.05 nM reversed the inhibition effect of **1**, the results are entirely consistent with an interaction of μ- opioid receptors. Furthermore, in a competitive study performed with morphine (a preferential μ- opiate recetor alkaloid ligand) and DAGO (a highly μ-selective opioid peptide), peptide **1** appears to be 150- fold more active than morphine (Table 1) and as active as DAGO. The strong inhibition of the spontaneous contractions of rabbit jejunum by peptide **1**, also indicated an interaction with δ-opioid receptors since this effect was totally blocked by naltrindole, an

established δ-opioid receptor antagonist, at an equimolecular concentration. Moreover, in comparison to peptides **2** and **3**, compound **1** appeared to be moderately more active as an inhibitor than either of the deltorphins, which were comparable to each other as to activity (Table 1 A and B).

**Table 1 A**

In vitro characterization of [Dmt1]deltorphin B and related peptides

| Compound | Ki Nm | | Kiμ /Kiδ |
|---|---|---|---|
| | μ | δ | |
| (1)[Dmt[1]]deltorphin B | 1,01 ± 0,06 (3) | 0,13 ± 0,027 (3) | 7,7 |
| (2)Deltorphin B | 638 ± 55 (5) | 0,12 ± 0,03 (6) | 5317 |
| (3)Deltorphin C | 272 ± 50 (11) | 0,24 ± 0,06 (6) | 1135 |
| Morphine | / | / | |
| DAGO | 1,51±0,18 (8) | 376,3 ± 81,8 (4) | 0,004 |

**Table 1 B**

| Compound | | $ED_{50,Nm}$ | |
|---|---|---|---|
| | | Guinea- pig ileum | Rabbit jejunum |
| (1) [Dmt[1]]deltorphin B | | 300 (216-353) | 360 (297-395) |
| (2) Deltorphin B | | / | 850 (775-915) |
| (3) Deltorphin C | | / | 730(665-794) |
| Morphine | | 43μM (38-52) | / |
| DAGO | | 22,0 (18,3-24,9) | / |

## Antinociception

The results obtained with the hot plate test indicated that 10 min after icv administation, peptide **1** induced a significant and dose- related analgesia in the mouse (Table 2), which lasted for the entire experimental period

(45 min). In a comparative study with morphine, peptide **1** was 180-fold more potent thant this narcotic opiate alkaloid, thus confirming the observations on the interaction with μ-opioid receptors with the in vitro bioassays (Table 1).

These observations were further confirmed with the tail flick test, in which peptide **1** had an analgesic activity comparable to that demonstrated in the hot plate test. Whereas peptide **2** was about **3** times more active than peptide **1**, all three deltorphin analogues **(1-3)** were about 300 to over 800- fold more analgesically potent than morphine (Table 2). A concurrent study then considered the effects of naltrindole (δ-opioid receptor antagonist), naloxone (μ-opioid receptor antagonist), and H-Dmt-Tic-Ala-OH (DTA), a new highly selective δ-opioid receptor antagonist [**46**] on the analgesic effect induced by peptides **1** and **3** (Table 3). While DTA and naltrindole (0,05 nM, icv) produced no measurable antinociception, both antagonized the antinociceptive action during co-administration of peptides **1** or **3** in the tail flick test (Table 3). Identical treatment with reported δ receptor antagonists failed to affect antinociception in the hot plate test (Table 3). On the other hand, pretreatment with naloxone (0.05 nM icv), which does not produce antinociception, antagonized the activity of peptide **1** in both the hot plate and tail flick tests (Table 3), but it antagonized peptide **3** in the tail flick test and not in the hot plate test (Table 3).Statistical analysis comparing data of Tables 2 and 3 showed significant ($P<0.01$) differences between peptides tested alone and the same in the presence of each antagonists except for deltorphin C in the hot plate test versus deltorphin C plus each antagonist in the hot plate test.

**Table 2**

In vitro antinociception activity of [Dmt] deltorphin B and related peptides

| Compound | Hot Plate Test | | Tail Flick test | |
|---|---|---|---|---|
| | i.c.v. $ED_{50}$nM | Rel. Prot. | i.c.v. $ED_{50}$nM | Rel. Pot. |
| (1)[ Dmt[1] ]deltorphin B | 0,016 (0,011-0,030 | 180 | 0,011 (0,0087-0,023) | 300 |
| (2) Deltorphin B | 0,021 (0,016-0,042) | 140 | 0,0038(0,0016-0,008) | 840 |
| (3) Deltorphin C | 0,035 (0,020-0,051) | 80 | 0,017 (0,01-0,021) | 190 |
| Morphine | 2,88 (1,75-3,9) | 1 | 3,18 (2,9-3,7) | 1 |

The peptides subtly modified in the side chain at N-terminus residue yielded an opioid with high dual affinities to both δ- and μ-opioid receptor types. At present, it appears that the replacement of the Tyr residue by the innatural Dmt analogue in the δ- selective opioid agonist, deltorphin B, produced a peptide with high affinity for both μ- and δ-opioid receptors, thereby yielding a δ-opioid receptor ligand which, to all intents and purposes, lacks δ-opioid receptor selectivity.

While the high affinity for the μ-opioid receptor was confirmed in the *in vitro* pharmacological assay (guinea- pig ileum), *in vivo* antinociception apparently failed to correlate with high μ-opioid receptor activity; however, the activity profile of peptide **1** [DMT[1]] deltorphin B was more closely related to that of the δ- selective opioid peptides **2** (deltorphin B) and **3** (deltorphin C).

In summary, [Dmt[1]] deltorphin B possesses remarkable agonist activity and exerts effects on both μ- and δ-opioid receptors *in vitro* and *in vivo*, and also that the newly discovered opioid tripeptide antagonist, DTA, exerts antagonist activity primarily toward the δ- opioid receptor, in keeping with *in vitro* data.

On conformational point of view the activity of the analogues including DMT in deltorphin B can be explained on the basis of a conformation of the N-terminal portion of deltorphin B where the rotation of the Tyr residue is sensibly reduced.

**Table 3**

Co- administration of, or pretreatment with, H- Dmt- Tic- Ala- OH (DTA), naltrindole (NTI) or naloxone (NAL) on the $ED_{50}$for antinociception produced by i.c.v. Dmt deltorphin B **(1)** and deltorphin C **(2)** n the hot plate test and tail flick test

Peptide

Hot plate test, $ED_{50}$ Nm

| | plus DTA | plus NTI | plus NAL |
|---|---|---|---|
| 1) | 0.097 (0.065 - 0.11) | 0,067 (0.042 - 0,081) | 1.76 (1.23 - 1,97) |
| 2) | 0.053 (0.031 - 0.083) | 0.042 (0.016 - 0.072) | 0.055 (0.01 - 0,073) |

Tail Flick test, $ED_{50}$, nM

| | plus DTA | plus NTI | plus NAL |
|---|---|---|---|
| 1) | 2.7 (2.9 - 3.1) | 1.9 (1.3 - 2.1) | 1.58 (1.1 - 1.84) |
| 2) | 2.3 (1.9 - 2.9) | 1.8 (1.1 - 2.3) | 0.77 (0.65 - 0.83) |

## Role of the TIC Moiety

The synthesis of an antagonist with high δ- selectivity and without the substitution of the basic nitrogen of the tyramine moiety was performed for the first time inserting a Tic (1,2,3,4-tetrahydroisoquinoline-3-carobxylic acid) moiety in the second position of a dermorphin related peptide Tyr-Tic-Phe-NH2 (dubbed TIP) and Tyr-Tic-Phe-Phe NH2 (dubbed TIPP).

Conformational studies of the Tyr-Tic analogs showed that the antagonism displayed by all [Tyr-$Tic^2$] peptides can be attributed to the relative arrangement of the two aromatic rings of Tyr and Tic, i.e. to a specific two-residue message domain represented by the sequence Tyr-Tic. The spatial relationship of these rings is very similar to the characteristic 90° arrangement assumed by the corresponding rings of several δ selective naltrindole derivatives described by Portoghese and coworkers [48]. This interpretation led to the synthesis of the first opioid dipeptide, Tyr-Tic-Phe-$NH_2$ [43], and to a series of simple and very potent δ selective antagonists. In fact, Tyr-Tic-Phe-$NH_2$ itself behaves as a δ selective antagonists [43] and even more significantly introduction of Tic in the second position of the sequence converts enkephalin, a non-selective agonist, and dermorphin (a μ-selective agonist) into δ selective antagonists [49]. The view of a specific "antagonist message" was subsequently substantiated by the discovery of ultraselective δ antagonists containing the Tyr-$(Me)_2$-Tic message [46,50] and by the design of a rigid antagonist lacking the basic charge of tyramine [51].

The conformations of [Tyr-Tic] and/or [Tyr-$(Me)_2$-Tic] peptides are greatly influenced by the conformational preferences of Tic. Since the amide nitrogen is part of a six-membered ring similar to that of pipecolic acid, the population of conformers containing a *cis* peptide bond are comparable to those of conformers containing trans peptide bonds. In addition, the Tic side chain conformation is confined to the single value of $\chi^1$ imposed by ciclization, a circumstance drastically different form the conformational freedom typical of aromatic aminoacid residues. Thus, the conformation accessible to Tyr-Tic peptides, notably the C1b+ cis conformer we previously identified [43] as a likely δ-selective bioactive conformation, are forbidden or severely disfavoured for opioid peptides containing an aromatic amino acid residue in the second position.

### EFFECTS OF TIC INSERTION IN DYNORPHIN A

Substitutions of Tic residue in the N-terminal region of DYN were made to ascertain whether it could alter their k-receptor agonist characteristics, converting the resulting peptides into an antagonist.

Tic replacement of $Gly^2$ in DYN A-(1-13)-$NH_2$ (**1**) or DYN A-(1-11)-$NH_2$ (**2**) yielded potent $\delta$ or $\mu$ antagonism. These findings are in agreement with the observation that $Tic^2$ substitutions in dermorphin (a $\mu$-selective agonist [52-53]) and [$Leu^5$]enkephalin (a $\delta$-agonist [52]) produced peptides with $\delta$ and $\mu$ antagonism, suggesting that Tic in the second position of opioid agonists might represent a key factor in eliciting antagonism.

However, Schmidt et al. [53] reported that [$Tic^2$]deltorphin C retained partial agonist activity *in vitro* in the MVD bioassay, whereas a deltorphin C octapeptide analogue, [$Tic^2$, $Phe^{3,4}$, $Asp^5$, $Val^{6,7}$, $Gly^8$-$NH_2$], was an antagonist with more $\delta$-receptor selective properties. Thus, the overwhelming preponderance of data confirm and substantiate that Tic in the second position of opioid peptides produces compounds with $\delta$ antagonism [52, 54-58].

Furthermore, the substitution of methyl groups at the 2' and 6' position of Tyr (Dmt) yielded Dmt-Tic- peptides (**3**) endowed with dramatically increased $\delta$-affinity, comparable to the $\delta$-affinity observed with deltorphins [59-63]. On the other hand, $\mu$-affinity of **3** rose 20-fold relative to **1** and **2**, while k-affinity increased over 40-fold in guinea pig brain membranes, 20-fold in rat brain synaptosomes compared to **2** and approximately 40-fold for both membrane preparation relative to **1**. Inclusion of the Tyr-D-Ala-Phe message domain (**4**) essentially eliminated opioid interaction with k-receptors, whereas $\delta$- and $\mu$-affinities were only moderately altered in comparison to DYN (1-11) -$NH_2$ (Table 4).

In the RJ bioassay, **1-3** antagonized the potent $\delta_{d2}$-opioid receptor agonist deltorphin B with a $pA_2$ ranging from 7.25 to 8.12. The agonist activity of **4** on GPI was due only to the interaction with $\mu$-receptors rather than k-receptors because **1-3** which are k-antagonists, failed to modify the IC50 of **4** in the same tissue (data not shown).

Analogues **1-3** exhibited antagonist activity on GPI ($\mu$ and k receptors) and RJ ($\delta$-receptors), while **4** was full agonist on both tissues (Table 4). In the GPI assay, peptides **1-3** antagonized the k-agonist U-50,488 with weaker activity than **2** which is a potent antagonist ($pA_2$=8,33). Although an apparent discrepancy occurred between **2** and **3** in receptor binding (Table 4) and functionally bioassays (Tables 4,5,6A and B), the data for each peptide are consistent for a given assay (i.e., **2** weakly interacted with $\delta$, $\mu$, and k-opioid receptors while the opposite was true for **3**). The anomalous behaviour noted previously by other investigator [64-66] between receptor affinity and bioassay may be due to several factors. One very likely possibility is alternative splicing in k- receptor mRNA [67] or alternated transcription start sites [68] that could produce multiple k- receptor variant in a tissue specific manner an account for the differential recognition of pharmacologically defined receptor subtypes. Other modifications related to receptor conformation that should be

considered include changes initiated by interaction of G-coupled proteins with the intracellular loops and differences in phosphorylation of the C-terminal region which would affect the geometry of the receptor ligand–binding domain to differentially affect opioid receptivity [69]. Furthermore, extracellular cations could also affect both peptide and receptor structures to cause a differential shift in the observed response between tissue and isolated membrane receptors.

Another suggestion that the overall 3-D shape and charge of the peptide ligand could dictate binding [70] was postulated by Crescenzi et al [71]. They proposed that the discrepancy in the receptor binding and bioactivity is directly related to the shape of the peptide ligand in the solution since the combined effects in entropic gain and the amounts of rotamer 3g- relative to 3t have approximately the same orders of magnitude in difference as observed between δ-receptor binding and bioactivity.

**Table 4**

Receptor binding data 1-4 and related peptides

| Peptide | Ki values delta | Ki values mu | k receptor binding (Ki, nM) | |
|---|---|---|---|---|
| | | | Rat | Guinea pig |
| 1.(tic$^2$)DYN-A-(1-13-)-$NH_2$ | 24,3±8,9(4) | 85,4±17,2(5) | 178,1±36,6 (4) | 118,0±11(4) |
| 2.(tic$^2$)DYN-A-(1-11)-$NH_2$ | 30,0±8,4(4) | 91.7±17.8 (5) | 91.7±18.9 (4) | 145.6±19 (4) |
| 3.($Dmt_1$, $Tic_2$)DYN-A-(1-11)-$NH_2$ | 0,39±0.07(4) | 4.15±1.3 (4) | 4.29±0.97 (4) | 3.32±0.49(4) |
| 4. (D-Ala,des- $Gly_3$)DYN-A-(1-11)-$NH_2$ | 39,9±10,3(4) | 39.2±11.0 (5) | 22.45±4.6.40 (3) | 1.435±106(4) |
| DYN-A-(1-11)-NH2[a] | 10.66±0.67 | 2.71±0.96 | / | / |
| Tyr- Tic- $NH_2$[b] | 166±48(4) | 28.712±3.30 5 (7) | / | / |
| (Tic$^2$,Leu$^5$) enkephalin[c] | 94±21(4) | 4.620±529(3) | / | / |
| (Tic$^2$)dermorphin[c] | 2.4±0,7(4) | 204±42(4) | / | / |

## Effects of Tic and Dmt Insertion In Message Domain

The rationale in designing peptides with the substitution of methyl groups at the 2' and 6' position of Tyr (Dmt) Dmt-Tic-OH (DTOH) and Dmt-Tic-Ala-OH (DTAOH), was to minimize the message domain of opioid peptides while maintaining activity. Thus, a class of highly potent and selective opioid di- and tripeptide δ antagonist [72] were synthesized

whose physiochemical properties might be amenable to passage through the BBB.

The design of DTOH peptide was performed on the basis of an 1H-NMR solution conformation of DTOH that supported the idea of DTOH similar to naltrindole (a nonapeptide delta antagonist) and suggested a viable binding conformation at the delta opioid receptor [73]. DTOH and DTAOH could be much more rigid inside the receptor with the methyl groups of Dmt favoring a better fit of the tyramine moiety in the T subsite of the opioid receptor (46 ,73].

**Table 5**

Functional bioassay data for 1-4 and related peptides

| Peptide | Guinea pig ileum | | | Rabbit jujunum | | |
|---|---|---|---|---|---|---|
| | $pA_2$ | Kc, nM | $IC_{50}$ nM | pA2 | Kc, nM | $IC_{50}$ nM |
| 1.($tic^2$)DYN-A-(1-13-)-$NH_2$ | 6,34±0,9 | 457 | / | 7,35±0,7 | 44,7 | / |
| 2.($tic^2$)DYN-A-(1-11)-$NH_2$ | 8,33±0,5 | 4,68 | / | 7,63±0,9 | 234 | / |
| 3.($Dmt_1$, $Tic_2$)DYN-A-(1-11)-$NH_2$ | 7,04±0,7 | 91,2 | / | 8,12±1,3 | 7,59 | / |
| 4.(D-Ala,des- $Gly_3$)DYN-A-(1-11)-$NH_2$ | / | 77 | / | / | 340 | / |
| DYN-A-(1-11)-NH2[a] | / | / | 2,88±0,2 8 | / | / | 13,09±2, 15 |
| Tyr- Tic- $NH_2$[b] | / | / | >10,000 | 6 | / | / |
| ($Tic^2$,$Leu^5$) enkephalin[c] | / | / | >10,000 | 6,5 | / | / |
| ($Tic^2$)dermorphin[c] | / | / | >10,000 | 7,5 | / | / |

**Table 6 A**

Analytical properties of 1-4

| Peptide | K' values[a] | | | | | |
|---|---|---|---|---|---|---|
| | I | II | $MH^+$ | Y | A | G |
| 1 | 5,27 | 6,03 | 1706 | 1,02 | / | 0,98 |
| 2 | 6,48 | 6,82 | 1464 | 0,97 | / | 1,02 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 7,21 | 7,76 | 1492 | / | / | 0,96 |
| 4 | 4,52 | 5,04 | 1319 | 1,05 | 0,98 | / |

**Table 6 B**

Analytical properties of 1-4

| Peptide | Amin Acid Analyses[b] | | | | | |
|---|---|---|---|---|---|---|
| | F | L | R | I | P | K |
| 1 | 1 | 1,93 | 2,87 | 0,92 | 1,01 | 2,08 |
| 2 | 1 | 1,03 | 3,02 | 0,95 | 0,97 | 1,04 |
| 3 | 1 | 0,97 | 2,91 | 1,01 | 0,92 | 1,07 |
| 4 | 1 | 1,07 | 2,94 | 1,03 | 0,96 | 1,03 |

The good fit of Dmt for the subsite might force the carboxyl group into close contact with the anion site of the $\mu$ receptor, explaining the high $\delta$ selectivity of these compounds [72]. Furthermore lipophilic aminoacids and water solubility imply a potential to transit the brain capillary endothelium.

Previous experiments performed *in vitro* with receptor binding assay [46, 74] mouse vas deferens (MVD) and guinea-pig ileum (GPI) [72] showed a marked delta opioid receptor preference.

**Table 7**

Antagonistic effect of DTOH and DTAOH after central (A) or (B) systemic administration

| Compound | A<br>Tail Flick Test<br>i.c.v. $ED_{50}$nM | B<br>Talk flick test<br>i.c.v. $ED_{50}$nM |
|---|---|---|
| Deltorphin II | 5 | 5 |
| DTOH+DEL II | 20 | 25 |
| DTAOH+DEL II | 22 | 5 |

Although receptor binding assay (brain membranes) revealed that DTOH and DTAOH exhibited high affinity and extraordinary selectivity for the $\delta$ opioid receptor and pharmacological experiments *in vitro* (MVD and GPI) further indicated their $\delta$ antagonist activity [72, 74] DTOH and DTAOH, showed a different behaviour against DEL antinociception, depending on the route of the administration used.

Both DTOH and DTAOH if injected centrally were able to antagonize DEL antinociception. On the contrary, after systemic administration only DTOH was able to antagonize DEL antinociception whereas DTAOH failed (Table 7).

The different behaviour after systemic administration of di- (DTOH) and tripeptide (DTAOH) treatment could reflect different pharmacokinetic of these two molecules containing a tyrosine surrogate, Dmt and a constrained phenylalanine, Tic.

DTOH is an active $\delta$-opioid dipeptide antagonist upon peripheral administration due to a combination of its unique physiochemical properties (lipophilic, water-solubility, stability).

**Role Of Phe$^2$ In μ-Selectivity.**

Many experimental evidences suggest that in opioid peptides the second position is strategic to orient the selectivity of the biological response. As previously reported the insertion of Tic residue in 2 position of the message sequence plays a basic role in driving δ selectivity.

The conformational preferences of analogs containing an aromatic residue (Xaa) in second position have been examined, i.e. Phe, Tyr, Trp or His, since it is likely that other relative arrangements confer to the peptides good opioid activity and a different selectivity.

The starting choice of the systematic search was Phe for the second residue (Xaa), because Tic was originally derived from this aminoacid residue. Instead of Tyr, Tyr-(Me)$_2$ was used as the first residue for all peptides, since it ensures a far better binding without departing too much from the constitution of the parent residue (Tyr). As already done in the design of the $\delta$ selective antagonists containing the Tyr-Tic message [75], in order to limit the synthetic efforts, a conformational analysis was performed by means of energy calculation of peptides containing the initial Tyr-(Me)$_2$-Phe sequence and the resulting energy minima were compared with appropiate rigid compounds. To have a meaningful comparison with the Tyr-(Me)$_2$-Phe sequence, a rigid mold with two aromatic rings was chosen. Many synthetic k opioids that have fairly low k/$\mu$ selectivity were used: in particular 6N-cinnamoyl-$\beta$-naltrexamine (CNX) for which the ratio of (Ki)$\mu$ is 0.2/0.07, i.e. slightly in favor of $\mu$ [76] , and ICI 199441 [77], which is more k selective.

A complete conformational search was done using intervals of 10° for all relevant internal rotation angles, i.e. $\chi^1{}_1$, $\psi_1$ $\phi_2$, $\psi_2$, $\chi^1{}_2$. Other internal

coordinates were minimized at each step. $\psi_1$ and $\Phi_2$ were chosen as most representative to describe the conformational preferences of the dipeptides, since they are the central rotation angles.
The energy map of Tyr-$(Me)_2$-Phe-$NHCH_3$ as a function of $\psi_1$ and $\phi_2$ shows one broad minimum centered approximately at 160/-80 (a). The energy map of Tyr-$(Me)_2$-D-Phe-$NHCH_3$ on the other hand shows three distinct minima of comparable energy centered at 150/60 (I), 150/60 (II) and –60/90 (III). None of these minima (a, i-iii) gave a satisfactory overlap with MeNTI. This finding may be viewed as an indirect confirmation that the bioactive conformation for $\delta$ selective dipeptides corresponds to the cis C1b+ conformer of Tyr-Tic $NH_2$, which however is not energetically accessible to the Tyr-$(Me)_2$-Phe-$NHCH_3$. On the other hand, minima (III) and to a lesser extent (II) have a fairly good overlay with the molecular model of CNX whereas minima (I) and to a lesser (II) have a fairly good overlay with the molecular model of ICI 199441. The fact that the fit is no perfect, particularly at the basic nitrogen and the second aromatic ring (distinct from that of tyramine), hints that high agonist activity may be prevented whereas antagonism is still likely [51].

Based on this conformational analysis the simple dipeptides Tyr-$(Me)_2$-D-Phe-$NHCH_3$ and Tyr-$(Me)_2$-Phe-$NHCH_3$ and two analogs containing the same messages but a longer, hydrophobic address sequence, i.e. Tyr-$(Me)_2$-D-Phe-Gly-Val-Val-$NH_2$ and Tyr-$(Me)_2$-Phe-Gly-Val-Val-$NH_2$ were synthesized. Table 8 summarizes the binding data and *in vitro* activity of these peptides. For comparison, the binding data of the corresponding D-Tyr-$(Me)_2$-Phe and D-Tyr-$(Me)_2$-D-Phe peptides are also reported.

All compounds show some preference for $\mu$ vs $\delta$ opioid receptors, and in all pentapeptides have better opioid receptor binding than the corresponding dipeptides, i.e. C-terminal elongation with hydrophobic residues (Val-Val) improves binding at both $\mu$ and $\delta$ opioid receptors.

On the other hand it is very interesting to observe that, similar to what we have recently reported for $\delta$ selective antagonists [75], a simple dipeptide sequence can behave as a kind of "antagonist message". In fact, although all compounds are inactive as a agonists in the GPI assay at concentrations $>10^{-4}$ M, consistent with binding data the two compounds with a L-Tyr-$(Me)_2$-D-Phe chirality message have $pA_2$s ranging from 6.5 to 7.3, depending on the $\mu$ agonist used. Intrinsic activity does not change from the dipeptides, Tyr-$(Me)_2$-D-Phe-$NH_2$ to the pentapeptide Tyr-$(Me)_2$-D-Phe-Gly-Val-Val-$NH_2$ in the stimulated GPI tissue.

On the other hand, all compounds are inactive in the tissue containing $\delta$ opioid receptors (RJ) at concentrations $>10^{-4}$ M. these compounds can be considered neither $\delta$ agonists nor $\delta$ antagonists since the typical values for RJ are an IC50 of 730 nM in the case of $\delta$ antagonist naltridole (unpublished results). This behaviour is particularly surprising for the

Tyr-(Me)$_2$-D-Phe di and pentapeptide that bind to $\delta$ receptors with Ki values of 15.5 and 2.32 nM respectively (Table 8).

**Table 8**

Binding and functional bioactivity of [Tyr(Me)$_2$.Phe] peptides

| Peptide | Ki/Nm $\delta$ | Ki/nM $\mu$ | GPI[b] |
|---|---|---|---|
| Tyr(Me)$_2$-Phe-NH$_2$ | 386.5 ± 52 (3) | 209.6 ± 48 (3) | i |
| d-Tyr(Me)$_2$-Phe-NH$_2$ | 1977 ± 412 (5) | 866.4 ± 141 (4) | i |
| Tyr(Me)$_2$-d-Phe-NH$_2$ | 15.5 ± 1.1 (4) | 65.3 )3( 4.0 ± | 7,2; 7,0[c];6.5[d] |
| d-Tyr(Me)$_2$-d-Phe-NH$_2$ | 2543 ± 334 (4) | 501± 92 (4) | 4,9 |
| Tyr(Me)$_2$-Phe-G-V-V-NH$_2$ | 118.3 ± 22 (4) | 75.3 ± 8.8 (4) | 5,7 |
| d-Tyr(Me)$_2$-Phe-G-V-V-NH$_2$ | 246.6 ± 55 (5) | 113.8 ± 18 (3) | 5 |
| Tyr(Me)$_2$-d-Phe-G-V-V-NH$_2$ | 2.32 ± 0.5 (5) | 0.53 ± 0.08 (3) | 7,3; 7,2[c]; 6.7[d] |
| d-Tyr(Me)2-d-Phe-G-V-V-NH2 | 22.3 ± 5,6 (5) | 2.1 ± 0.4 (3) | 5,2 |

The $\mu$ opioid antagonist activity of the dipeptide is remarkable also considering that the only know $\mu$ antagonist of peptide structure, i.e. H-D-Phe-c-(Cys-Tyr-D-Trp-Arg-Thr-Pen)Thr-NH$_2$ (pA$_2$=6.4÷7.9), a cyclic octapeptide derived from somatostatin [24], contains the Tyr-D-Trp motif in its sequence. The fairly small $\mu$ selectivity is not too surprising if one takes into account the role of a further locus of interaction (different from the two aromatic residues) present in longer peptides [78, 79]. However, the recent discovery of a very selective $\mu$ agonist tetrapeptide [80] hints that the search for short selective peptides is still realistic. Owing to its simple chemical constitution Tyr-(Me)$_2$-D-Phe-NH$_2$ looks to be a very promising $\mu$ antagonist both as a lead structure and for practical applications.

**Role of D-glucopyranosyl moiety in the pharmacological activity of Deltorphin C and dermorphin.**

One major avenue of investigation in the field of opioid agonist and antagonist molecules involves the development of opioid analogues that penetrate the blood-brain-barrier (BBB) In fact inadequate blood-brain barrier (BBB) permeation can be responsible for low analgesic potencies.

As previous reported, *Phyllomedusa* amphibians secrete two major classes of opioid peptides from their skin glands. [6, 8-9] dermorphins (dermal morphine-like substances) and deltorphins (delta-specific opioids). They are originally designated as the prototypes of respectively δ and μ agonist activity. DER and DEL as well as their synthetic analogues are generally resistant to enzymatic degradation, [34-35] and showed potent antinociceptive effects following intracerebroventricular administration, but only moderate or negligible central actions after systemic administration in mice and rats [36-38]. The synthesis and the biological activity of Deltorphin C and dermorphin with a D-glucopyranosyl moiety was undertaken in order to take advantage of the glucose transporter [43] and enhance the passage through the BBB. These analogues were characterised by a D-glucopyranosyl moiety beta-O-glycosidically linked to a Thr4 or Thr7 side chain.
Opioid receptor affinities of glycopeptides were determined by displacement of selective radioligands from rat brain membrane synaptosomes. The δ and μ opioid receptor binding sites were specifically labeled using $[^3H]$[D-Pen$^{2,5}$]enkephalin (DPDPE) and $[^3H]$[DAla$^2$,MePhe$^4$,Gly-ol$^5$]enkephalin (DAGO), respectively (Table 9). Furthermore, the bioactivity of compounds listed in Table 9 were tested *in vitro* in two isolated organ preparations, guinea pig ileum (GPI) and rabbit jejunum (RJ), run as described previously [29, 81] (Table 10).
Competitive-binding experiments (Table 9) indicate that in the DEL C peptides, the presence of a D-glucopyranosyl moyety linked to Thr side chains differentially affects affinities ($K_i$) and selectivity ($K_i/K_i$). In fact, the O-glycosylation at Thr$^7$ (compounds **1** and **2**) produces a moderate decrease in δ and in μ receptor affinities, while the introduction of the identical carbohydrate unit at Thr$^4$ (compounds **3** and **4**) is very detrimental, particularly at δ sites. A similar trend was also observed for DER-related glycopeptides **5-8**. In fact, analogues **5** and **6** modified at position 7 possess affinity and selectivity characteristics close to those of DER, whereas compounds **7** and **8** modified at position 4 display low affinities.
These results reinforced earlier observations indicating that modifications at the C-terminal part in DEL C and DER permit expression of high δ and/or μ receptor affinities, whereas the changes accompanying modifications at position 4 depend on the chemical nature or bulkiness of the side chain [9,25,31]. The present data indicate that the loss of backbone flexibility imposed by the bulky glycosylated Thr$^4$ in DER and DEL C peptides may presumably limit the opioid binding [31]. The binding properties for δ and μ opioid receptors for all of glycopeptides were consistent with the biological activity as tested by GPI and RJ bioassays. These experiments indicated that DER glycopeptides **5** and **6** reduced the electrically induced contractions of guinea pig ileum while DEL C glyco derivatives **1** and **2** failed to cause significant inhibition up

to micromolar concentrations (Table 10). Since naloxone at a concentration of 0.05 nM reversed the inhibition effect of **5** and **6**, the results are entirely consistent with an interaction of μ opioid receptors. On the other hand, the inhibition of the spontaneous contractions of rabbit jejenum by glyco compounds **1** and **2** indicated an interaction with δ opioid receptors since these effects were totally blocked by naltrindole, [82] an established δ opioid receptor antagonist, at an equimolecular concentration.

**Table 9 Receptor Binding Properties of Glycopeptides**

| No. | Peptide Ligand | Kiδ(nM) | Kiμ (nM) | Kiδ/kiμ |
|---|---|---|---|---|
| 1 | Tyr-D-Ala-Phe-Asp-Val-Val-Thr[ β-d-Glc(OAc)$_4$ ]-Gly-NH$_2$ | 1.30 ± 0.3 | 634.9 ± 94 | 0.002 |
| 2 | Tyr-D-Ala-Phe-Asp-Val-Val-Thr (β-d-Glc)-Gly-NH$_2$ | 1.55 ± 0.5 | 578.9 ± 38 | 0.002 |
| 3 | Tyr-D-Ala-Phe-Thr[β-D-Glc(OAc)$_4$]-Val-Val-Gly-NH$_2$ | 2110 | 1520 | 1.3 |
| 4 | Tyr-D-Ala-Phe-Thr(β-D-Glc)-Val-Val-Gly-NH$_2$ | 9500 | 1993 | 4.8 |
| | Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH$_2$ (deltorphin C) | 0.15 ± 0.03 | 147 ± 29 | 0.001 |
| 5 | Tyr-D-Ala-Phe-Gly-Tyr-Pro-Thr[β-D-Glc(OAc)$_4$.]-Gly-NH$_2$ | 86.8 ± 14.7 | 0.46 ± 0.06 | 190 |
| 6 | Tyr-D-Ala-Phe-Gly-Tyr-Pro-Thr(β-D-Glc)-Gly-NH$_2$ | 67.0 ± 22 | 0.29 ± 0.02 | 231 |
| 7 | Tyr-D-Ala-Phe[β-D-Glc(OAc)$_4$]Tyr-Pro-Ser-NH$_2$ | 22600 | 4400 | 5 |
| 8 | Tyr-D-Ala-Phe-Thr(β-D-Glc)-Tyr-Pro-Ser-NH$_2$ | 1760 | 1700 | 1 |
| 9 | Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$ (deltorphin C) | 82.5 ± 16.1 | 0.28 ± 0.03 | 295 |

*In vitro* active glycopeptides were also tested *in vivo* by a mouse tail-flick assay [83] after intracerebroventricular (icv) and subcutaneous (sc) injections as described previously [84]. In this test, icv administration of DEL C analogues **1** and **2** displayed significant antinociceptive action (Table 10) comparable to that of the parent DEL C.

The analgesic profile of the latter compound is in agreement with the trend previously observed for [D-Ala$^2$]deltorphin II [37]. DER-related glycopeptides **5** and **6** were about 5-fold more analgesically potent than **1** and **2** but were also 2 orders of magnitude less active than DER.

This is quite unexpected since glycopeptides **5** and **6** showed binding properties and *in vitro* activities (Table 9 and 10) rather similar to that of DER.

**Table 10** In Vitro and in Vivo Activities of Glycopeptides

| | $IC_{50}$ (nM) | | tail - flick test[b] | |
|---|---|---|---|---|
| | GPI | RJ | i.c.v. $ED_{60}$ mM | a.c. $ED_{60}$ |
| Compound | | | | |
| 1 | | 890 ± 81 | 10.6 (6.6 - 23.2) | / |
| 2 | | 995 ± 89 | 11.3 (7.1 - 22.9) | 96.4 (51.8 - 168) |
| 3 | nt | > 4000 | nt | |
| 4 | nt | > 4000 | nt | |
| 5 | 22.1 ± 2.4 | | 2.8 (1.9 - 3.8) | 88.9 (46.5 - 151) |
| 6 | 3.1 ± 0.2 | | 1.9 (0.9 - 2.7) | 0.53 (0.38 - 1.31) |
| 7 | > 4000 | | nt | nt |
| 8 | 3400 | | nt | nt |
| DEL C | | 510 ± 62 | 5.0 (4.8 - 6.1) | / |
| DER | 2.1 ± 0.18 | | 0.015 (0.009 - 0.080) | 3.1 (1.9 - 5.9) |
| morphine | | | 3.2 (2.9 - 3.7) | 10.9 (7.0 - 18.8) |

The discrepancies between binding or bioassay data and icv analgesia may be due to a different degree of nonspecific adsorption of peptides in various tissues or to reduced intrinsic activity of glycopeptides at the central opioid receptors. Since DER and Tyr-D-Ala-Phe-Gly-Tyr-Pro-Thr-Gly-$NH_2$ possess enzymatic stability [23, 34] and since a glycopeptide has generally enhanced resistance to brain enzymes, [85,86] all examined compounds can be expected to be equally stable against enzymolysis under the conditions of the tests, and thus, a different extent of peptide degradation in the various tissues can be ruled out as a factor explaining the differences between different bioassay data. Naltrindole (0.05 nM, icv) antagonized the antinociceptive action during coadministration of DEL C peptides, while pretreatment with naloxone (0.05 nM, icv) antagonized the activity of DER derivatives (data not shown). Evaluation of the antinociceptive effects of glycopeptides after sc injection in mice (Table 10) revealed additional interesting differences due to structural modifications to DEL C and DER peptides. The fully acetylated glycopeptide **1**, like DEL C, was unable to produce antinociception up to 150 mg/kg, while deacetylated glycopeptide **2** showed low but significant analgesia. Apparently, this result can be

interpreted to be in agreement with the predictions made on the basis of the glucose transporter(s) concept [87] and with the trend previously observed for glycoenkephalins [85].
Such an interpretation seems to be supported by the comparison of analgesic characteristics that pertain to DER analogues. In fact, glycopeptide **6** displayed remarkable sc analgesic activity: it was 20 times as potent as morphine and, particularly, 170 and 6 times more potent than its parent acetylated glycoanalogue **5** and dermorphin, respectively. This finding is even more remarkable when one takes into account that unglycosylated peptide Tyr-D-Ala-Phe-Gly-Tyr-Pro-Thr-Gly-$NH_2$ retains only 20-30% of DER sc analgesia, [23] and considering that icv antinociception of glycopeptide **6** was significantly much lower than that of DER (Table 10).
In summary, an appropriate attachment of beta-D-glucose unit in deltorphin or dermorphin peptides give rise to glyco analogues retaining high δ or μ selectivity and remarkable activity *in vivo*. In particular, as systemic antinociceptive agents, the glucoside-bearing compounds **2** and **6** are more potent than the parent unglycosylated peptide counterparts. This probably indicates that peripheral glycopeptides possess (a) an adeguate diffusion, from subcutaneous sites into the bloodstream, which can be attributed to their evident water solubility, and (b) a high blood to brain rate of influx which may be due, at least in part, to the glucose transporter GLUT-1 [87]

# CONCLUSIONS

The results of the present rewiev have demonstrated that:

**a**) The replacement of the Tyr residue by the innatural Dmt analogue in the δ- selective opioid agonist, deltorphin B, produced a peptide with high affinity for both μ- and δ-opioid receptors, thereby yielding a δ-opioid receptor ligand which, to sall intents and purposes, lacks δ-opioid receptor selectivity. While the high affinity for the μ-opioid receptor was confirmed in the *in vitro* pharmacological assay (guinea- pig ileum), *in vivo* antinociception apparently failed to correlate with high μ-opioid receptor activity; however, the activity profile of peptide **1** was more closely related to that of the δ- selective opioid peptides **2** and **3**. Similarly, the analgesic activity of the δ- opioid-selective enkephalin analogue [D-Ser (O-tert-butyl)$^2$, Leu$^5$] enkephalyn- Thr(O-tert-butyl)$^6$ (BUBU) and [Dmt$^1$] DPDPE is mediated through different receptor types: the μ-opioid receptor in the case of BUBU and an unknown δ-opioid receptor subtype for the latter peptide. In summary, our results indicate that [Dmt$^1$] deltorphin B possesses remarkable agonist activity and exerts effects on both μ- and δ-opioid receptors *in vitro* and *in vivo*, and also that the newly discovered opioid tripeptide antagonist, DTA, exerts antagonist

activity primarily toward the δ-opioid receptor, in keeping with *in vitro* data.

**b)** The Tic replacement of $Gly^2$ in DYN A analogues yielded potent δ- (**2**) and k-opioid antagonism (**3**), while **2** exhibited the highest binding affinities to δ, μ and k receptors. These findings further support the observation that $Tic^2$ substitutions in dermorphin (a μ-selective agonist and [$Leu^5$]enkephalin (a δ-agonist) produced peptides with δ-antagonism, suggesting that Tic in the second position of opioid agonists might represent a key factor in eliciting antagonism. However, Schmidt et al. [44] reported that [$Tic^2$]deltorphin C retained partial agonist activity *in vitro* in the MVD bioassay, whereas a deltorphin C octapeptide analogue, [$Tic^2$, $Phe^{3,4}$, $Asp^5$, $Val^{6,7}$, $Gly^8$-$NH_2$], was an antagonist with more δ-receptor selective propeties. Thus, the overwhelming preponderance of data confirm and substantiate that Tic in the second position of opioid peptides produces compounds with δ antagonism.

**c)** Both DTOH and DTAOH if injected centrally were able to antagonize DEL antinociception. On the contrary, after systemic administration only DTOH was able to antagonize DEL antinociception whereas DTAOH failed. The different behaviour after systemic administration of di-(DTOH) and tripeptide (DTAOH) treatment could reflect different pharmacokinetic of these two molecules containing a tyrosine surrogate, Dmt and a constrained phenylalanine, Tic. A detailed conformational analysis of DTOH in solution shows a highly selective non peptide δ antagonist reported by Portoghese [32] that is active upon peripheral administration [11]. Also, DTOH and DTAOH could be much more rigid inside the receptor with the methyl groups of Dmt favouring a better fit of the tyramine moiety in the T subsite of the opioid receptor. The good fit of Dmt for the subsite might force the carboxyl group into close contact with the anion site of the μ receptor, explaining the high δ selectivity of these compounds. Our results provide evidence that DTOH is an active δ-opioid dipeptide antagonist upon peripheral administration due to a combination of its unique physiochemical properties (lipophilic, water-solubility, stability). This peptide could be clinically and therapeutically useful because, while opioid peptides generally antagonize an antinociceptive action, following systemic injection.

**d)**The μ opioid antagonist activity of the dipeptide is remarkable also considering that the only know μ antagonist of peptide structure, i.e. H-D-Phe-c-(Cys-Tyr-D-Trp-Arg-Thr-Pen)Thr-$NH_2$ ($pA_2$=6.4÷7.9), a cyclic octapeptide derived from somatostatin [24], contains the Tyr-D-Trp motif in its sequence. The fairly small μ selectivity is not too surprising if one takes into account the role of a further locus of interaction (different from the two aromatic residues) present in longer peptides. However, the recent

discovery of a very selective $\mu$ agonist tetrapeptide hints that the search for short selective peptides is still realistic. Owing to its simple chemical constitution Tyr-$(Me)_2$-D-Phe-$NH_2$ looks to be a very promising $\mu$ antagonist both as a lead structure and for practical applications.

e) An appropriate attachment of beta-D-glucose unit in deltorphin or dermorphin peptides give rise to glyco analogues retaining high $\delta$ or $\mu$ selectivity and remarkable activity *in vivo*. In particular, as systemic antinociceptive agents, the glucoside-bearing compounds **2** and **6** are more potent than the parent unglycosylated peptide counterparts. This probably indicates that peripheral glycopeptides possess (a) an adeguate diffusion, from subcutaneous sites into the bloodstream, which can be attributed to their evident water solubility, and (b) a high blood to brain rate of influx which may be due, at least in part, to the glucose transporter GLUT-1.

## REFERENCES

[1] Herz A. Opioids I. Springer Verlag: New York, **1993**.

[2] Hughes, J.; Smithg, T.; Kosterlitz, H.N.; ,Fothergill. L.A.; Morgan, B.; Morris, R.H.; *Nature* London **1975**;258,577-

[3] Terenius, L.:, Wahlstrom, A.; *Life Sci* **1975**, 16, 1759-

[4] Ughes, J.;. *Brain Res* **1975**, 88,285-

[5] Zadina, J.E.; Hackler, L.; Ge, L.; Kastin, A.; *Nature* (London), **1997**, 386, 499-

[6] Erspamer, V.; *Int. J. Dev. Neurosci.* **1992**, *10*, 3-30.

[7] Bryant, S.D.; Attila, M.; Salvadori, S. ; *Environ. Health Perspect.* **1994**, *102*, 648-654.

[8] Montecucchi, P. C.; De Castiglione, R.; Piani, S.; Gozzini, L.; Erspamer, V. *Int. J. Pept. Protein Res.* **1981**, *17*, 275-283.

[9]Lazarus, L. H.; Salvadori, S.; Santagada, V.; Tomatis, R.; Wilson, W. E. , *J. Med. Chem.* **1991**, *34*, 1350-1359.

[10] Jaffe J.M.;, Martin W.R.; In *The Pharmacologian Basis of Therapeutics;* Gilman, A., Rall, J., Nies, A.; Taylor, P.; editors.; Pergamon, New York; **1990**; Vol. 485, pp.

[11] Rowbatham, M.C.; *Ann Neurol*, **1994,** 35, S46.

[12] Hammack, J.E., Lorpinzi, C.L.; *Mayo Clin Proc*, **1994**, 69, 384-

[13] Hammond, D.L.; In *Current & Emerging Iussues of Cancer Pain: Research and Practice.* Chapman, C.R., ed Raven New York, **1993**, 175-

[14] Froelich, J.C., Li T-K. In *Recent Developments in Alcoholism, Volume II: ten Years of Progress.* Galanter M, ed. New York: Plenum, **1993**,187-

[15] Arakawa, K., Akami, T., Okamoto, M., Akioka, K., Kakai, I., Oka, N., Negase, H., *Transplant Proc.*, **1993**, 25, 738-

[16] Schad, C.A., Justice, J.B. Jr, Holtzman, S.G.,. *Eur J Pharmacol.* **1995**, 275, 9-.

[17]Heyman, J.S.; Vaught, J.L.; Ruffa, R.B.; Porreca, F.;.,*Trends Pharmacol. Sci,* **1988**, 9,134-

[18] Galligan, J.J.; Mosberg, H.I.; Hursi, R.; Hruby, V.J.; Burst, T.F.; J *Pharmacol, Exp. Ther.* **1984,** 268,607-.
[19] Hruby, V.J.; Gehring, C.A.; *Med. Res. Rev* **1989**, 9-343.
[20] Quian, X.; Kover, K.E.; Shenderovic, M.D.; Lou, B.S.; Misika, A.; Zalewska, T.; Horvath, R.; Davis, P.; Bilsky, E.J.; Porreca, F.; Yamamur, H.J.; Hruby, V.J.; *J Med Chem,* **1994**, 37,1746.
[21] De Castiglione, R.; Faoro, F.; Perseo, G.; Piani, S.; Santangelo, F.; Melchiorri, P.; Falconieri, Erspamer, G.; Erspamer, V.; Guglietta, A. *Peptides* **1981**, *2*, 265-269.
[22] Darlak, K.; Grzonka, Z.; Janicki, P.; Gumulka, S. W. *Peptides* **1984**, *5*, 687-689.
[23] Marastoni, M.; Salvadori, S.; Balboni, G.; Marzola, G.; Degli Uberti, E. C.; Tomatis, R. *Int. J. Pept. Protein Res.* **1986**, *28*, 274-281.
[24] Sagan, S.; Amiche, M.; Delfour, A.; Camus, A.; Mor, A.; Nicolas, P. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 726-732.
[25] Attila, M.; Salvadori, S.; Balboni, G.; Bryant, S. D.; Lazarus, L. H. *Int. J. Pept. Protein Res.* **1993**, *42*, 550-559.
[26] Schmidt, R.; Chung, N. N.; Lemieux, C.; Schiller, P. W. *Regul. Pept.* **1994**, *54*, 259-260.
[27] Misika, A.; Lipkowski, A. W.; Fang, L.; Knapp, R. J.; Davis, P.; Kramer, T.; Burks, T. F.; Yamamura, H. I.; Carr, D. B.; Hruby, V. J. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1290-1297.
[28] Lazarus, L. H.; Salvadori, S.; Attila, M.; Grieco, P.; Bundy, D. M.; Wilson, W. E.; Tomatis, R. *Peptides* **1993**, *14*, 21-28.
[29] Salvadori, S.; Bryant, S. D.; Bianchi, C.; Balboni, G.; Attila, M.; Lazarus, L. H. *J. Med. Chem.* **1993**, *36*, 3748-3756.
[30] Heyl, D. L.; Dandabathula, M.; Kurtz, K. R.; Mousigian, C. *J. Med. Chem.* **1995**, *38*, 1242-1246.
[31] Lazarus, L. H.; Bryant, S. D.; Salvadori, S.; Attila, M.; Jones, L. S. *Trends Neurosci.* **1996,** *1*, 31-35 and references cited therein
[32] Portoghese, P.S.; Sultana, M.; Nagase, H.; Takemori, A.E.; *J Med Chem.*, **1988,** 31,281-
[33] Calderon, N.S.; Rothman, R.B.; Porreca, F.; Flippen- Anderson, J.L.; Mcnutt, R.W.; Xu H.,Smith, L.E.; Bilsky, E.J.; Davis, P.; Rice, K.C.; *J Med Chem* , **1994,** 37,2125-
[34] Scalia, S.; Salvadori, S.; Marastoni, M.; Bortolotti, F.; Tomatis, R. *Peptides* **1986**, *7*, 247-251.
[35] Marastoni, M.; Tomatis, R.; Balboni, G.; Salvadori, S. *Farmaco* **1991**, *46*, 1273-1279.
[36] Negri, L.; Falconieri, Erspamer, G.; Severini, C.; Melchiorri, P.; Erspamer, V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7203-7207.
[37] Jang, Q.; Mosberg, H. I.; Porreca, F. *Life Sci. P. L.* **1990**, *47*, 43-47.
[38] Negri, L.; Noviello, L.; Noviello, V. *Eur. J. Pharmacol.* **1996**, *296*, 9-16.
[39] Weber, S.J.; Greene, D.L.;.,Sharma, S.D.; Yamamura, H.I.;. Kramer, T.H.; Burks, T.F.; Hrúby, V.J Hersh, L.B.; Davis, T.P.; *.J.Pharmacol. Exp. Ther.* 259 1109-1117;
[40] W.A. Banks, K.L. Audus And T.P. Davis Peptides, **1992**, 13, 1289-1294.
[41] N. Bodor , L. Rokai, W.M. Wu, H. Farag, S. Jajalagadda, M. Kawamura And J. Simpkins, Science, **1992,** 257, 1698-1700

[42] Polt, R.; Porreca, F.; Szabo', L.Z.; Bilsky, E.J.; Davis, P.; Abbruscato, T.J., Davis, T.P.; Harvath, R.; Yahamura, H.I.; Hruby, V.I. *Proc. Natl. Acad. Sci. USA* , 91, 7114-71181.
[43] Temussi, P.A.; Salvadori, S.; Amodeo, P.; Bianchi, C.; Guerrini, R.; Tomatis, R.; Lazarus, L.H.; Picone, D.; Tancredi, T.; *Biochem. Biophys. Res. Commun.,* **1994** 198, 933-939
[44] Heyman, J.S.; Vaught, J.L.; Ruffa, R.B.; Porreca, F.; *Trends Pharmacol. Sci.* **1988** 9,134-
[45] Erspamer, V.; Melchiorri, P.; Erspamer, G.; Negri, L.; Corsi, R.; Severini, C.; Barra, D.; Simmaco, M.; Kreil, G.; *Proc.Natl, Acad. Sci. USA* **1989**,86,5188.
[46] Salvadori, S.; Attila, M.; Balboni, G.; Bianchi, C.; Bryant, S.D.; Crescenzi, O.; Guerrini, R.; Picone, D.; Tncredi, T.; Temussi, P.A.; Lazarus, L.H.; *J Mol. Med,* **1995**, 1, 678-689
[47] Bryant, S. D.; Salvadori, S.; Guerrini, G.; Lazarus, L.H.; *J Med Chem* **1996** .
[48] Portoghese, P.S.; Sultana, M.; Nagase, H.; Takemori, A.E.; *Eur. J.Pharmacol.* **1992** 218, 195-196.
[49] Tancredi, T.; Salvadori, S.; Amodeo, P.; Picone, D.; Lazarus, L.H.; Bryant, S.D.; Guerrini, R.; Marzola, G.; Temussi, P.A.; *Eur J.Biochem.* **1994**, 224,241-247.
[50] Amodeo, P.; Baldoni, G.; Crescenzi, O.; Guerrini, R.; Picone, D.; Salvadori, S.; Tancredi, T.; Temussi, P.A.; *FEBS Lett.* **1995,** 377,363-367.
[51] Crescenzi, O.; Fraternali, F.; Picone, D.; Tancredi, T.; Balboni, G.; Guerrini, R.; Lazarus, L.H.; Salvadori, S.; Temussi, P.A.; *Eur J. Biochem* **1997**, 247,66-73.
[52] Tancredi, T.; Salvadori, S.; Amodeo, P.; Picone, D.; Lazarus, L.H.; Bryant, S.D.; Guerrini, R.; Marzola, G.; Temussi, P.A.;. *Eur J Biochem*, **1994**, 224, 241.
[53] Schmidt, R.; Chung, N.N.; Lemieux, C.; Schiller. P.W.; *Regulatory Peptides* , **1994**, 54:259.
[54] Schiller, P.W.; Nguyen, T.M.D.; Weltrowska, G.; Wilkes, B.C.; Marsden, B.J.; Lemieux, C.; Chung, N.N.; *Proc Natl Acad Sci U.S.A.* **1992**, 89, 11871.
[55]Schiller, P.W.; Weltrowska, G.; Nguyen, T.M.-D.; Wilkes B.C.; Chung, N.N.; Lemiex, C.J Med Chem 1993, 36, 3182.
[56] Temussi, P.A.; Salvadori, S.; Amodeo, P.; Guerrini, R.; Tomatis, R.; Lazarus, L.H.; Picone, D.; Tancredi, T.; *Biochem Biophys Res Commun* **1994**, 198, 933-
[57] Salvadori, S.; Attila, M.; Balboni, G.; Bianchi, C.; Bryant, S.D.; Crescenzi, O.; Guerrini, R.; Picone, D.; Tancredi, T.; Temussi, P.A.; Lazarus, L.H.; *Mol Med* , **1995**, 1, 678.
[58] Schiller, P.W., Nguyen, T.M.-D.; Weltrowska, G.; Wilkes, B.C.; Marsden, B.J.; Schmidt, R.; Lemieux, C.; In Hodges RS,Smith JA, editors. *Peptides: Chemistry and Biology*. Leiden:ESCOM.**1994**, 483.
[59] Erspamer V. *Int. J., Devel. Neurosci* **1992,** 10:03.
[60] Marastoni, M.; Tomatis, R.; Balboni, G.; Salvatori, S.; Lazarus, L.H.; *Farmaco,* **1991**, 46, 1273.
[61] Lazarus, L.H.; Salvadori, S.; Santagada, V.; Tomatis, R.; Wilson, W.E., *J. Med Chem*, **1991**, 34, 1350.
[62] Lazarus, L.H.; Salvadori, S.; Balboni, G.; Tomatis, R.; Wilson, W.E.; *Med Chem*; **1992**, 35, 1222.

[63] Schiller, P.W.; Weltrowska, G.; Nguyen, T.M-D.; Wilkes, B.C.; Chung, N.N; Lemieux, C.; *J Med Chem*, **1992**, 35, 3956.
[64] Schiller P.W.; Weltrowska, G.; Nguyen, T.M-D.; Wilkes, B.C.; Chung, N.N.; Lemieux, C.; *J Med Chem*, **1992**, 35, 3956.
[65] Schiller, P.W.; Nguyen, T.M-D.; Chung, N.N.; Lemieux, C.; *J Med Chem* **1989**;32:698.
[66] Fang, L.; Knapp, R.J.; Horvath, R.; Matsunaga, T.O.; Haaseth, R.C; Hruby, V.J.; Porreca, F.; Yamamura, H.I.; *J. Pharmacol Exper Ther*, **1994**, 268:836.
[67] Belkowski, S.M.; Zhu, F.; Liu-Chen, L-Y.; Eisenstein, T.K; Adler, M.W.; Rogers, M.W.; *J Neuroimmunol,* **1995**, 62, 113.
[68] Yakovlev, A.G.; Kruger, K.E.; Faden, A.I.; *J Biol Chem*, **1995**, 270, 6421.
[69]Chang, K-J.; Hazum, E.; Killian, A.; Cuatrecasas, P.; *Mol Pharmacol,* **1981**, 20, 1.
[70] Moran, S.; Ren, RX-F.; Rumney, I.Y.S.; Kool, E.T.; *J Am Chem Soc*, **1997**,119, 2056.
[71] Crescenzi, O.; Fraternali, F.; Picone, D.; Tancredi, T.; Balboni, G.; Guerrini, R.; Lazarus, L.H.; Salvadori, S.; Temussi, P.A., *Eur Biochem.*, **1997**, 247, 66, 35.
[72] Lazarus, L.H.; De Castiglione, R.; Guglietta, A.; Wilson, W.E.; *J. Biol. Chem.*, **1989**, 264, 3047-3050
[73]. Capasso, A.; Giannuario, A.; Loizzo, A.; Pieretti S.; Sorrentino, L.; *Life Sci.*, **1992** 51, PL139-143
[74] Amodeo, P.; Balboni, G.; Crescenzi O.; Guerrini, R.; Picone, D.; Salvatori, S.; Tancredi T.; Temussi P.A.; *FEBS Lett.*, **1995**, 377 363-367
[75] Temussi, P.A.; Salvadori, S.; Amodeo, P.; Guerrini, R.; Tomatis, R.; Lazarus, L.H.; Picone, D.; Tancredi, T.; *Biochem .Biophys. Res. Commun.* **1994**,198, 933-939.
[76] Derrick, I.; Moynihan, H.A.; Broadbear, J.; Woods, I.H.A.; Lewis, J.W.; *Bioorg. Med.Chem.Lett.* **1996**, 6, 167-172.
[77] Costello, G.F.; James, R.; Shaw, J.S.; Slater, A.M.; Stutchbury, N.C.J.; *J.Med.Chem.*, **1991**, 34,181-189.
[78] Garzon, J.; Sanchez- Blazquez, P.; Hollt, V.; Lee, N.M.; Loh, H.H.; *Life Sci.*, **1983**, 33,291-294.
[79] Mansour, A.; Hoversten, M.T.; Taylor, L.P.; Watson, S.J.; Akil, H.) *Brain Res.* **1995**, 700,89-98.
[80] Zadina, J.E.; Hacker, L.; Ge, Lin-J. Kastin, A.J.; *Nature*, **1997**, 386,499-502.
[81]Valeri, P.; Morrone, L. A.; Romanelli, L. *Br. J. Pharmacol.* **1992**, *106*, 39-44.
[82] Portoghese, P. S.; Sultana, M.; Takemori, A. E. *Eur. J. Pharmacol.*, **1988**, *146*, 502-507.
[83] Janssen, P. A. J.; Memegeers, C. J.; Dony, J. D. *Arzneim.-Forsch.* **1963**, *13*, 502-507.
[84] Guerini, R.; Capasso, A.; Sorrentino, R.; Anacardio, R.; Bryant, S. D.; Lazarus, L.-H.; Attila, M.; Salvadori, S. B. *Eur. J. Pharmacol.* **1996**, *302*, 37-42.
[85] Polt, R.; Porreca, F.; Szabo, L. Z.; Bilsky, E. J.; Davis, T. P.; Horvath, R.; Yamamura, H. I.; Hruby, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7114-7118.
[86] (a) Horvat, S.; Horvat, J.; Vargadefterdarvocic, L.; Pavelic, K.; Chung, N. N.; Schiller, P. W. *Int. J. Pept. Protein Res.* **1993**, *41*, 339-404. (b) Vargadefterdarovic, L.; Horvat, S.; Schiller, P. W. *Int. J. Pept. Protein Res.* **1992**, *39*, 12-17.

[87] Bell, G. I.; Burant, C. F.; Taked, J.; Gould, G. W. *J. Biol. Chem.* **1993**, *268*, 19161-19164.

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# EARTHWORM FIBRINOLYTIC ENZYME

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**ABSTRACT:** A group of fibrinolytic enzymes, secreted by the alimentary tract of earthworm such as *Lumbricus rubellus* and *Eisenia fetida*, are called earthworm fibrinolytic enzymes (EFEs). They are in characteristics of alkaline trypsin-, chymotrypsin- and elastinase-like serine proteases. These enzymes, having relatively broad substrate specificity, degrade fibrinogen and fibrin directly, and also activate plasminogen (PLg). Their activities can be measured based on the amidolytic activity for several chromogenic substrates, fibrin plate and light scattering of the fibrin turbidity. EFEs have been made as an orally administered fibrinolytic agent. Their current clinical utilizations and some potential new applications will be discussed.

## INTRODUCTION

Earthworm has been used as a traditional medicine to treat clotting diseases in eastern countries for centuries. In *Compendium of Materia Medica* edited by a Chinese traditional medical doctor Shizhen Li (1518-1593), earthworm was described as a kind of drug prescribed for antipyretic and diuretic purposes in clinic. The dried earthworm prepared into powder, with a medicinal name: "Earth-dragon". So far, such a preparation is still used as a traditional folk remedy in China, Japan, Korea and other Asian countries. In 1878, Frédéricq [1] observed that an enzyme secreted from the alimentary tract of earthworm. In 1920, Keilin [2] isolated a kind of earthworm and found several proteases contained in the worm that could degrade casein, gelatine and albumin. Tens years later, Mihara separated a group of fibrinolytic enzymes (lumbrokinase, LK) from the extract of an earthworm *Lumbricus rubellus* [3,4]. However, this group of enzymes is now commonly called earthworm fibrinolytic enzymes (EFEs).

## ISOZYMES FROM DIFFERENT SPECIES

From 1980's, several research groups studied the components of EFE, independently [4,5,6]. Different purification methods were employed to isolate the enzymes, including sulfuric ammonia precipitation, gel filtration, ion exchanging, high-pressure liquid chromatography (HPLC)

and affinity chromatography. Several enzymes were isolated, such as earthworm powder enzyme (EPE) [1], earthworm fibrinolytic enzyme (EFE) [3,7,8], earthworm-tissue plasminogen activator (e-TPA) [9], earthworm plasminogen activator (e-PA) [10-15], component A of EFE (EFEa) [16,17] and biologically active glycolopoprotein complex (G-90) [18-23]. Different species of earthworms may have different structural and active components. Although the resultant components depend upon the purification methods, one enzyme may have different names, for instance F-II alias EFEa. However, the total number of components involved in fibrinolysis of an earthworm is still unknown.

## Isozymes From *Lumbricus rubellus*

The earthworm, commonly used for medicine, is *L. rubellus*. Mihara isolated and obtained three fractions (F-I, F-II and F-III) of fibrinolytic enzymes, from the homogenized extract [1,3,4]. Six isozymes were identified according to their further purification [24]. F-I and F-III could be subdivided into three (F-I-0, -I-1, and -I-2) and two (F-III-1 and -III-2) components, respectively, except F-II. The molecular mass of each isozyme (monomer) has been measured by several methods (Table **1**.). The isoelecctric point (*pI*) analysis displays weak acids of these isozymes. The biological functions (F-II and F-III-1) maximally exhibit around pH 9.0 at 50°C [7].

**Table 1. Molecular masses of earthworm fibrinolytic enzymes from *L. rubellus* ***

| | F-I-0 | F-I-1 | F-I-2 | F-II | F-III-1 | F-III-2 |
|---|---|---|---|---|---|---|
| **SDS-PAGE** | 43,000 | 40,000 | 38,000 | 36,000 | 27,000 | 24,000 |
| **Gel Filtration** | 24,000 | 26,000 | 28,000 | 28,000 | 38,000 | 38,000 |
| **Ionspray-MS** | 23,013 | 24,196 | 24,220 | 24,664 | 29,667 | 26,662 |

Mass is shown in Da. (* Refer to Nakajima, N.; *et al. Biosci. Biotech., Biochem.* **1993**, *57*, 1726-1730.)

## Isozymes From *Eisenia fetida*

Zhou *et al.* [5] found that at least seven components with fibrinolytic activity from earthworm *E. fetida*, Fig. (**1**). Three components are purified by fast protein liquid chromatography and proved to be homogenous on sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), with apparent molecular masses 30,000, 25,000 and 30,000 Da, respectively. These enzymes are stable at pH 5~9, but totally denaturated below pH 2.6.

In 1998, a plasminogen activator (e-PA, a dimer, 45,000 Da) was

separated from *E. fetida* [11]. The two constituting subunits (26,000 and 18,000 Da) hold together by hydrophobic interaction. The amino acid composition results show that the small subunit is absence of Cys residue but the large absence of Lys. This enzyme is also considered as a serine protease, and however, the two subunits exhibit different fibrinolytic activities individually when incubated on fibrin plate [11].

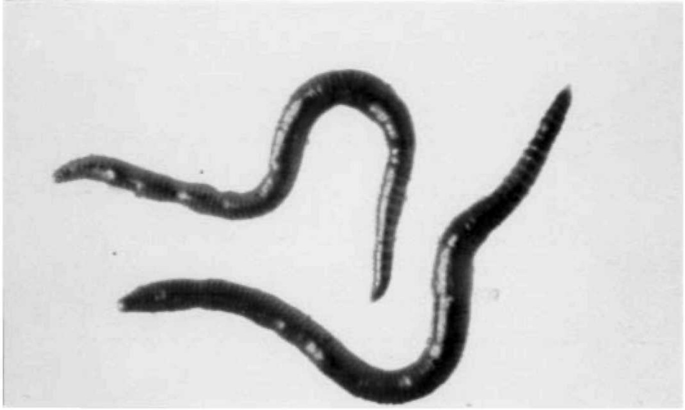

**Fig. (1).** Earthworm (*Eisenia fetida*)

## Isozymes From *Lumbricus bimastus*

In 2002, an up-to-date report by Xu *et al.* [25] shows that an extraction of fibrinolytic enzymes from *L. bimastus* contains three proteins with apparent molecular masses about 30,000, 29,000 and 28,000 Da in SDS-PAGE, respectively. According to the N-terminal sequences, a cDNA is obtained by reverse transcriptase PCR (RT-PCR). The cDNA is inserted in a vector and confirmed by sequencing. The results indicate that the fragment encodes a 242-amino-acid protein called $PV_{242}$ [26]. The whole amino acid sequence is 35% homologous to either F-III-1 or -III-2.

In earthworm's taxonomy, *Lumbricidae* are one of the largest families. *Lumbricidae*, as shown in Fig. (**2**) having the genus and species in three grades [27], is considered the recently evolved family, and it mainly distributes over Europe, America and Asia. Currently, *L. rubellus* (humus earthworm) and *E. fetida* (common brandling worm) can be raised and cultivated in large amount. In general, earthworm is used as a material for both experimental and productive purposes, such as environmental protection, agriculture production and biomedicine investigation. Anyway, they are getting more significant to involve in our daily life.

***Lumbricidae* ***

*Allolobophora*
*Allolobophora sp.*
*Aporrectodea*
*Aporrectodea rosea*
*Dendrobaena*
*Dendrobaena clujensis*
*Eisenia*
*Eisenia andrei*
*Eisenia fetida* (common brandling worm)
*Lumbricus*
*Lumbricus bimastus*
*Lumbricus castaneus*
*Lumbricus rubellus* (humus earthworm)
*Lumbricus terrestris* (common earthworm)
*Lumbricus sp.*
*Octolasion*
*Octolasion tyrtaeum*
*Octolasium*
*Octolasium lacteum*
Unclassified *Lumbricidae*
*Lumbricid sp. Samford*

**Fig. (2).** The taxonomic structure of earthworm *Lumbricidae* family.
* Refer to NCBI taxonomy database and the website:
http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi

# BIOCHEMICAL & PHARMACOLOGAL CHARACTERIZATION

## Structural Features

### *Primary Structure*

As shown in Table (**2**), the N-terminal sequences (24 residues) of the six isozymes from *L. rubellus* have been analyzed [24].

All the isozymes have a lot of overlapping identical residues and some regions of the sequences show similar characteristics to serine-like proteases, such as elastase and coagulation factor IX. The N-terminal sequences of F-III-1 and -III-2 are identical, and so are those of F-I-1 and F-I-2, respectively. So far, several complete reading frames (amino acid sequences) of the isozymes, F-III-1 and -III-2 (*L. rubellus*) [24,28], EFE

component A from *E. fetida* [16,17] identical to F-II (*L. rubellus*), and EFE-B (*L. bimastus*) have been sequenced, Fig. (**3**).

The isozymes are rich in both Asn and Asp, and comprise a few of Lys. From the results of sequencing, amino acid composition and immunological analyses [24], it is suggested these six isozymes are derived from at least four different genes. Recent studies show that some enzymes are glycosylated, which need further researching [29-31].

Moreover, some cDNA sequences were determined (see Genbank), such as Choi's in 1995 (U 25648 and U 25643), Sturzenbaum's in 1997 (AJ 223152). According the homologue analysis and sequence alignment, there could be some nonidentity homologs in sequences isolated from same earthworm species in different countries, this might be resulted from site-mutation occurred under dissimilar conditions.

```
           1        10        20        30       40         50        60        70
EFEa       VIGGTNASPGEFPWQLSQQRQSGSWSHSCGASLLSSTSALSASHCVDGVLPNNIRVIAGLWQQSDTSGTQ
F-III-1    MELPPGTKIVGGIEARPYEFPWQVSVRRKSSDSHFCGGSIINDRWVVCAAHCMQGEAPALVSLVVGEHDR
F-III-2    MELPPGKIVGGIEARPYEFPWQVSVRRKSSDSHFCGGSIINDRWVVCAAHCMQGESPALVSLVVGEHDSS
EFE-B      MLLLALASLVAVGFAQPPVWYPGGQCGVSQYSDAGDMELPPGTKIVGGIEARPYEFPWQVSVRRKSSDSH

           71       80        90        100      110        120       130       140
EFEa       TANVDSYTMHENYGAGTASYSNDIAILHLATSISLGGNIQAAVLPANNNDYAGTTCVISGWGRTDGTNN
F-III-1    SAASTVRQTHDVDSIFVHEDYNANTLENDVSVIKTSVAITFDINVGPICAPDPANDYVYRKSQCSGWGTI
F-III-2    AASTVRQTHDVDSIFVNENYDPRTLENDVSVIKTAIAITFDINVGPICAPDPANDYVYRKSQCSGWGTIN
EFE-B      FCGGSIINDRWVVCAAHCMQGESPALVSLVVGEHDSSAASTVRQTHDVDSIFVHEDYNGNTFENDVSVIK

          141        150      160       170        180        190       200       210
EFEa       LPDILQKSSIPVITTAQCTAAMVGVGGANIWDNHICVQDPAGNTGACNGDSGGPLNCPDGGTRVVGVTSW
F-III-1    NSGGICCPNVLRYVTLNVTTNQFCEDVYPLNSIYDDMICASDNTGGNDRDSCQGDSGGPLSVKDGSGIFS
F-III-2    SGGICCPAVLRYVTLNITTNAFCDAVYTSDTIYDDMICATDNTGMTDRDSCQGDSGGPLSVKDGSGIFSL
EFE-B      TVNAIAIDINVGPICAPDPANDYVYRKSQCSGWGTINSGGVCCPNVLRYVTLNVTTNAFCDDIYSPLYTI

          211       220       230       240        250        260       270       280
EFEa       VVSSGLGACLPDYPSVYTRVSAYLGWIGDNSR
F-III-1    LIGIVSWGIGCASGYPGVYSRVGFHAAWITDIITNN
F-III-2    GGIVSWGIGCASGYPGVYSRVGFHAGWITDTITNN
EFE-B      TSDMICATDNTGQNERDSCQGDSGGPLSVKDGSGIFSLIGIVSWGIGCASGYPGVYARVGSQTGWITDIITNN
```

**Fig. (3).** Some sequences of earthworm fibrinolytic enzymes.

EFEa is from *E. fetida* (GenBank database, AAM73677.1); F-III-1 (BAB40768.1) and F-III-2 (BAB40767.1) are from *L. rubellus*; EFE-B (AAL28118.1) is from *L. bimastus*.

**Table 2. N-terminal sequences (24 residues) of the six isozymes from *Lumbricus rubellus****

| Isozymes | N-terminal amino acid sequences |
|---|---|
| F-III-1 | I-V-G-G-I-E-A-R-P-Y-E-F-P-W-Q-V-S-V-R-R-K-S-S-D- |
| F-III-2 | I-V-G-G-I-E-A-R-P-Y-E-F-P-W-Q-V-S-V-R-R-K-S-S-D- |
| F-II | V-I-G-G-T-N-A-S-P-G-E-F-P-W-Q-L-S-Q-Q-R-X-S-G-S- |
| F-I-2 | I-I-G-G-S-N-A-S-P-G-E-F-P-W-Q-L-S-Q-T-R-G-G-S-H- |
| F-I-1 | I-I-G-G-S-N-A-S-P-G-E-F-P-W-Q-L-S-Q-T-R-G-G-S-H- |
| F-I-0 | V-V-G-G-S-D-T-T-I-G-Q-Y-P-H-Q-L-S-L-R-V-T-G-S-H- |

* Refer to Nakajima, N.; *et al. Biosci. Biotech., Biochem.*, **1993**, *57*, 1726-1730.

*Secondary Structure*

The secondary structures of F-III-1 [32], III-2 [33], F-II (EFEa) [34], and EFE-B (*L. bimastus*) [35] are predicted on the basis of their primary structures. The proteins have distinct predicted secondary structures, e.g. β-sheets, separated by α-helix, turn and coil, as shown in Table (**3**).

**Table 3. The secondary structures predicted by Double Prediction Method (DPM), Antheprot software**

| | F-III-1* | F-III-2* | EFEa** | EFE-B*** |
|---|---|---|---|---|
| Helix (%) | 7 | 4 | 6 | 5 |
| Sheet (%) | 40 | 41 | 30 | 40 |
| Turn (%) | 15 | 14 | 18 | 15 |
| Coil (%) | 37 | 41 | 46 | 39 |

The isozymes were from * *L. rubellus*, ***E. fetida* and *** *L. bimastus*, respectively.

The sequence alignment of EFEa shows that the sequence has high similarities compared to some related serine proteases with known structures: such as pocine pancreatic elastase (PPE) [36], human leukocyte elastase (HLE) [37], trypsin [38], chymotrypsin [39], the catalytic domain of U-plasminogen activator (u-PA) [40], tissue plasminogen activator (t-PA) [41] and plasmin [42]. The sequence identities of EFEa to other earthworm serine protease are also not significant: about 35% to F-III-1 and -III-2 [43], and 31% to earthworm chymotrypsinogen [44]. The highest sequence homology (44%) of EFEa is observed with lugworm (*Arenicola marina*) chymotrypsinogen [45], as shown in Table (**4**).

**Table 4. Comparison of homologous sequences with some serine proteases ***

| Proteases | Identity (%) |
|---|---|
| Pocine pancreatic elastase (PPE) | 36 |
| Human leukocyte elastase (HLE) | 28 |
| Trypsin | 35 |
| Chymotrypsin | 34 |
| U(t)-PA, Plasmin | 32 |
| F-III-1(2) | 35 |
| Earthworm chymotrypsinogen | 31 |
| Lugworm chymotrypsinogen | 44 |

* Refer to Tang, Y.; *et al. J. Mol. Biol.*, **2002**, *321*, 57-68.

*Tertiary Structure*

Crystallization and preliminary X-ray analyses of EFEa interestingly provide the structural information to understand the biological function [16]. The diffraction-quality single crystal was grown by the hanging drop vapor-diffusion technique with ammonium sulfate as a precipitant Fig. (**4**). As described by Tang *et al.* [16], the crystal belongs to the orthorhombic space group $p2_12_12_1$, with unit-cell parameters a = 40.6, b = 127.5, c = 129.2 Å and three molecules per asymmetric unit, with a $V_M$ of 2.24 Å 3 da-1, corresponding to a solvent content of 45.0%.

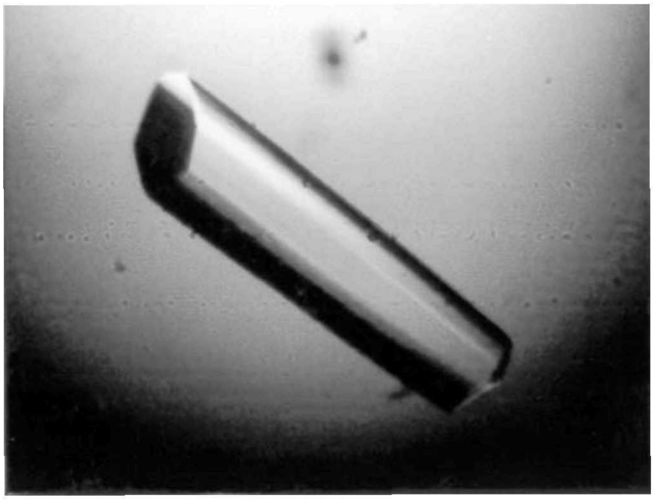

**Fig. (4). Crystallization of component A of EFE from *E. fetida* (homolog of F-II).**
This figure is kindly provided by Chang, W.R. *et al.*(see Tang, Y.; *et al. Acta Cryst.*, **2000**, *56*, 1659-1661.)

In the recent studies, the enzyme shows that the overall polypeptide fold of chymotrypsin-like serine protease possesses essential S1 specificity determinants characteristic of elastase using the multiple isomorphous replacement (MIR) method and refined to 2.3 Å resolution Fig. (**5**). Structure-based inhibitor modeling demonstrated that EFEa's S1 specificity pocket is preferable for elastase-specific small hydrophobic P1 residues, while its accommodation of long and/or bulky P1 residues is also feasible if enhanced binding of the substrate and induced fit of the S1 pocket are achieved [Fig. (**6**) shows the active sites of serine protease]. EFEa is thereby endowed with relatively broad substrate specificity, including the dual fibrinolysis. This structure is the first report of an earthworm fibrinolytic enzyme component, a serine protease originated from annelid worm [17].

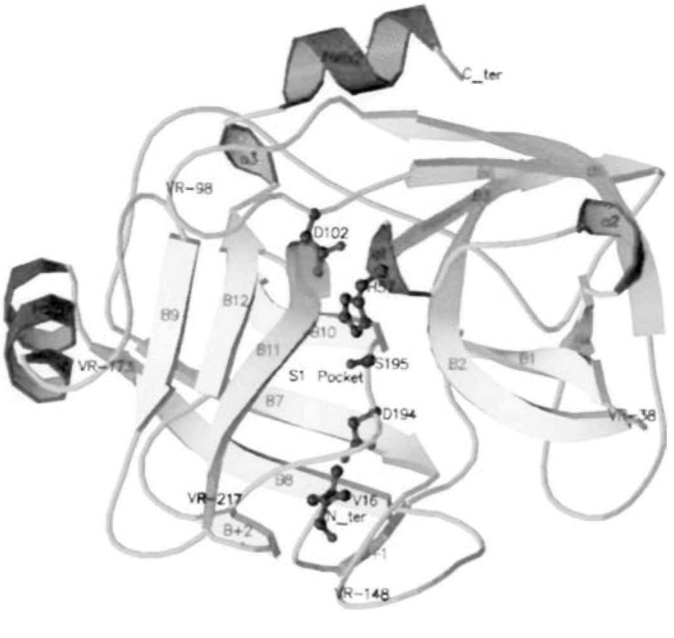


**Fig. (5).** Ribbon representation of F-II homolog from *E. fetida* structure in standard orientation. Strands are shown in yellow, helices in red, and loop regions in green. This figure is kindly provided by Chang, W.R. *et al.* (See Tang, Y.; *et al. J. Mol. Biol,* **2002**, *321*, 57-68.).

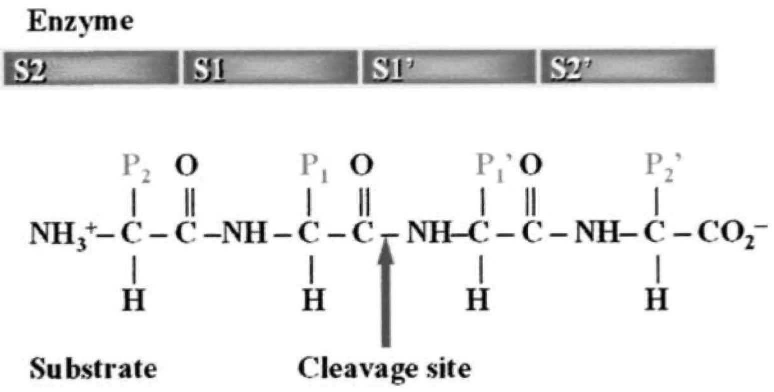


**Fig. (6).** The active site in serine protease.
Pn and Pn' represent the number of amino acid up- and down-stream of the cleavage site; Sn and Sn' are the binding groups at the active site of the serine protease.
Refer to Schechter, I.; Berger, A.; *Biochem. Biophys. Res. Commun.*, **1967**, *27*, 157-162.

## Activity Assays

Assay of the isozymers from *L. rubellus* is based on the enzymic reaction with various substrates. F-I and F-III are thought to represent a chymotrypsin-like and a trypsin-like protease, respectively [24]. F-II appears to act as a trypsin-like protease or an elastase. Interestingly, F-III-1 and -2, also with strong caseinolytic activity, have much higher fibrinolytic activity than plasmin as described by Mihara *et al.* [1,3]

### *Fibrin Plate*

Initially, fibrinolytic activity is measured by both plasminogen-rich and plasminogen-free fibrin plates [46,47]. When an individual earthworm is cut into pieces and placed on a plasminogen-rich fibrin plate, fibrinolytic activity can be detected at the anterior portion of the earthworm [3]. The average fibrinolytic activity of the 6-isozyme mixture is about 100 CU (plasmin unit) and 250 IU (urokinase unit) [3,24]. Although the fibrin plate is usually used to measure the activity, it is a time-consuming process (18~24 hr) indeed.

*Chromophoric Procedure*

Recently, an assay using chromophoric substrate (Chromozym TH) was developed [7]. When Chromozym TH is added, the absorbance at 405nm will increase, and the linear section from 15 to 30 sec is used to calculate the activity (international unit, one unit is defined as the specific activity required to convert 1 μM substrate/min/mg of enzyme), Fig. (**7**). The increasing absorbance is resulted from the *p*-nitroanilide released off the substrate during hydrolyzation. As compared with the fibrin plate method, this chromogenic assay is more rapid, convenient and reproducible. The specificity of the assay, however, is lower than that of fibrin-plate method because the chromogenic substrate could be hydrolyzed by some other proteases, for instance thrombin.

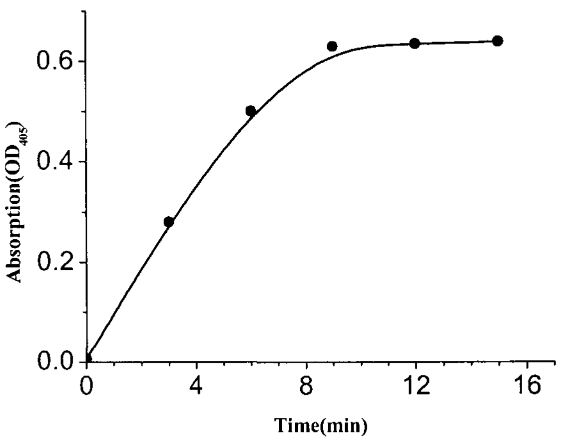


**Fig. (7).** Absorbance change of Chromozym TH at 405nm in the presence of F-II (See Zhou, J.; *et al. Protein and Peptide letters*, **1997**, *4*, 409-414.)

*Light Scattering*

Another method monitoring the conversion of fibrinogen into fibrin, a colloid, light scattering substance, is developed for the assay [48]. The scattering intensity of the thrombin-fibrin colloid exhibits the maximum at 480 nm. Changes in the scattering intensity follow a sigmoid curve with a relaxation phase and the maximum at 480 nm, as shown in Fig. (**8**). The intercept at the maximum slope, which is directly proportional to the concentration of thrombin as fibrinogen at a given concentration, is

employed to calculate the thrombin activity. EFE blocks the increase in the light scattering intensity, because the enzyme hydrolyzes both fibrinogen and fibrin. The amount of EFE is inversely proportional to the intensity. This method is rapid and reproducible. The comparison of these three methods is shown in Table (5).

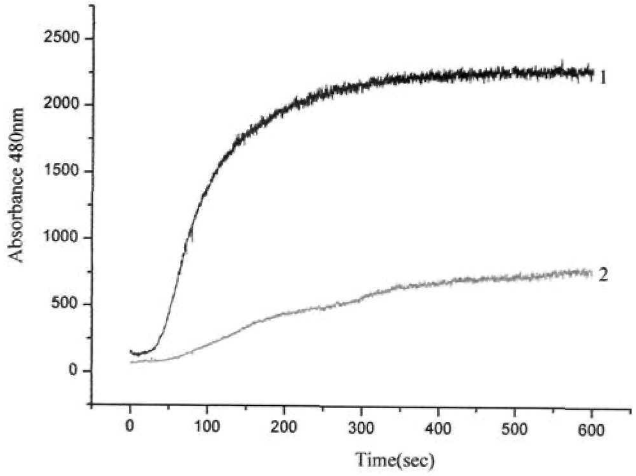


**Fig. (8). Changes in the light scattering intensity at 480nm during the assay of EFE with thrombin-fibrinogen solution.**
Curve 1: Mixture of fibrinogen and thrombin is used as control;
Curve 2: Fibrinogen is incubated with EFE for 8 min before thrombin is added.

**Table 5. Three assays of EFE**

| Assays | Fibrin plate | Chromophoric procedure | Light Scattering |
|---|---|---|---|
| **Materials** | Fibrinogen, thrombin, plasminogen and agar | chromophoric oligopeptides, for instance Chromozym-TH | Fibrinogen, thrombin system |
| **Principle** | Depending on the fibrinolytic area | *P*-Nitroanilide released from the substrate oligopeptide followed by absorbance increasing at 405nm | To block the scattering intensity resulted from fibrin colloid at 480 nm |
| **Time** | 18~24 hr | ~1 min | ~10 min |
| **Notation** | Time-consuming process | Rapid, convenient and reproducible, but lower specificity | Rapid and reproducible, lower specificity |

## EFE Inhibitors

EFEs are inhibited by a number of inhibitors, such as diisopropyl fluorophosphase (DFP) [24], soybean trypsin inhibitor (SBTI) [12,24], aprotinin [12,24], 1,6-hexamethylendiamine (HD) [49] and $\alpha_2$-macroglobulin ($\alpha_2$M) [50], etc. Nakajima *et al.* investigated some of the inhibitors when H-*D*-Phe-Pip-Arg-pNA is used as the substrate, as shown in Table (**6**). [24]. DFP completely inhibits the activity of all the isozymes (pH 7.2) at room temperature. F-III-1 and F-II are strongly inhibited by SBTI and aprotinin, but F-I-0, -I-1 and -I-2 are partially inhibited. Nevertheless, Tosyl-phenylalanyl chloromethylketose (TPCK), tosyl-lysyl-chloromethylketose (TLCK), ε-amino caproic acid (ε-ACA), elastatinal, EDTA and various metal ions, such as $Fe^{2+}$, $Cu^{2+}$ and $Mn^{2+}$ cannot be detected to affect the activity under the conditions [24]. On the basis of the specificity of substrates and inhibitors, EFE isomers are again exhibited the characteristics of alkaline serine-like proteases.

**Table 6. Effects of inhibitors on EFEs ***

| Inhibitors | Concentration | Residual activity (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | F-I-0 | F-I-1 | F-I-2 | F-II | F-III-1 | F-III-2 |
| None | - | 100 | 100 | 100 | 100 | 100 | 100 |
| DFP | 0.1mM | 1.0 | 1.0 | 1.0 | 0.1 | 0.1 | 0.1 |
| SBTI | 0.001mg/ml | 40 | 40 | 40 | 17 | 1.4 | 0.5 |
| Aprotinin | 0.001mg/ml | 85 | 85 | 84 | 25 | 0.3 | 0.7 |
| ε-ACA | 0.1mM | 90 | 90 | 90 | 90 | 90 | 90 |
| TPCK | 0.1mM | 90 | 90 | 100 | 100 | 100 | 100 |
| TLCK | 0.1mM | 80 | 100 | 80 | 80 | 75 | 85 |
| Elastatinal | 0.1mg/ml | 70 | 100 | 92 | 92 | 94 | 99 |
| EDTA | 1mM | 100 | 100 | 100 | 100 | 100 | 100 |

* Referred to Nakajima, N. *et al.*; *Biosci. Biotech. Biochem.*, **1993**, *57*, 1726-1730.

$\alpha_2$-Macroglobulin is present at a high concentration (approx. 2.0 μM) in blood plasma and inhibits all four classes of (cysteine-, serine-, aspartate- and metallo-) proteases [51,52]. The interaction between $\alpha_2$M and F-III-1 was investigated in the presence of Chromozym TH [50]. The fibrinolytic activity of F-III-1 decreased to 65% when incubated with $\alpha_2$M, while it decreases to 20% in the plasma under the same condition. The first order rate of inactivation of F-III-1 with $\alpha_2$M is similar to that with plasma, indicating that $\alpha_2$M may be the inhibitor initially binding to the enzyme in blood. Experiments in both the inhibition and the intrinsic fluorescence show that $\alpha_2$M binds to the enzyme mole by mole

equivalently, and support the view that $\alpha_2M$ conformational changes occur before EFE is inactivated. Furthermore, the $\alpha_2M$/EFE interaction may undergo a chelate irreversible inhibition. It suggests that $\alpha_2M$ may be one of the important inhibitors in the case of EFE absorbed in blood.

Although EFE is antagonized to serine protease inhibitors, immobilized EFE is hardly affected [52]. The resistance of immobilized enzyme to the inhibitors may be resulted from combination of the enzyme with the matrix and it stabilizes the spatial structure of the protein. The inhibitors could hardly induce the conformation of the active site into a proper transient structure that favors the interaction between the inhibitor and the enzyme.

## Fibrinolytic Mechanism and Cleavage Sites

EFEs have relatively broad substrate specificities, such as trypsin (cleavage site: Lys-X and Arg-X, X represents an amino acid) and chymotrypsin (cleavage site: Phe-X, Trp-X, Tyr-X and Leu-X), as shown in Fig. (9) [28]. F-III-2 cleaves β-amyloid 1-40 at six sites, and oxidized insulin B-chain at five sites and F-II digested them at six and ten, respectively [28].

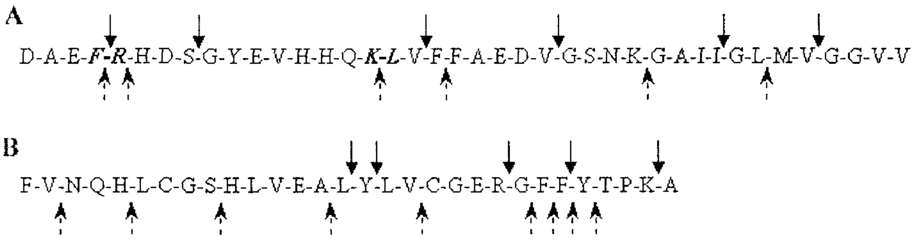


**Fig. (9).** A. The cleavage of β-amyloid 1-40 by F-III-2 (in line-arrow over the sequence) and F-II (in dot-arrow under the sequence) from *L. rubellus*, with trypsin (F-R in bold and italic), and chymotrypsin (K-L in bold and italic) as control.
B. The cleavage of oxidized insulin B chain by F-III-2 (in black arrow) and F-II (in dot arrow).
(Data are base on Nakajima, N.; *et al. Biosci. Biotech. Biochem.*, **1999**, *63*, 2031-2033.)

Mihara considered EFE having similar function to both plasmin and tissue plasminogen activator (t-PA) [3]. That is to say, it does not only degrade fibrin, but also partially activates PLg. In addition, some results indicate that F-II hydrolyzes creatine kinase (CK), immunoglobulin G (IgG) and bovine serum albumin (BSA) [53].

The immobilized F-II cleaves human plasminogen at four hydrolytic sites: $Lys_{77}$-$Arg_{78}$, $Arg_{342}$-$Met_{343}$, $Ala_{444}$-$Ala_{445}$ and $Arg_{557}$-$Ile_{558}$ (unpublished data of this lab). The last cleavage site ($Arg_{557}$-$Ile_{558}$) is the same as that of t-PA and urokinase (UK) [54-57], producing the active plasmin. F-II degrades α-A chain of fibrinogen at C-terminal region, (similar to human neutrophil elastase, HNE) [58], and the N-terminal region at the site of $Val_{21}$-$Glu_{22}$ (unpublished data of this lab). This site is different from that recognized by thrombin, and it produces soluble degraded fragments.

EFE interferes with thrombus formation in the early stages by digesting the adsorbed fibrinogen and other thrombogenic blot components. It is observable that the enzyme directly hydrolyzes thrombus *in vitro* [59], though such a hydrolysis needs further confirming *in vivo*. In addition, EFE inhibits fibrin to induce platelet aggregation by rapid fibrinolytic mechanism after fibrin net formation [60,61]. So far, we could pictorialize a diagram to show the mechanism of anticoagulation of EFE as follows [62-66], Fig. (**10**).

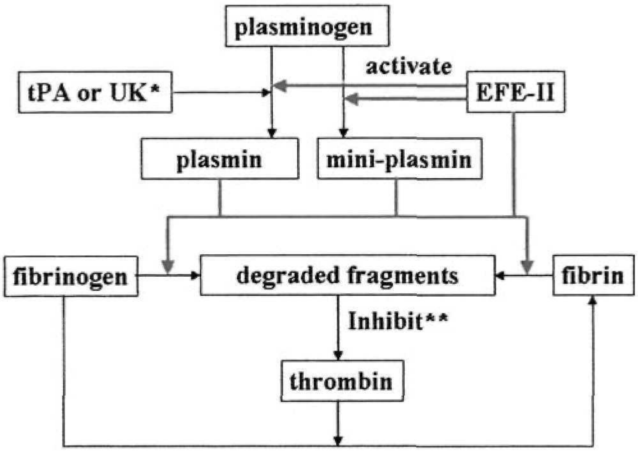


**Fig. (10). Diagram of anticoagulation of EFE from *L. rubellus*.**

Red line represents the effect of EFE

*Referred to Hoylaerts *et al. J. Biol. Chem.*, 1982, *257*, 2912-2919., and Wun *et al. J. Biol. Chem.*, **1982**, *257*, 3276-3283. **Referred to Standker *et al. Biochem. Biophys. Res. Commun.*, **1995**, *215*, 896-902.

# CLINICAL APPLICATIONS

## EFEs as a Novel Fibrinolytic Agent

Thrombosis is one of the most common and devastating diseases. Fibrinolytic enzymes are effectively in treating thrombosis. A variety of fibrinolytic enzymes, such as UK, streptokinase (SK), recombinant tissue-type plasminogen activator (rt-PA), staphylokinase (SAK) and recombinant prourokinase (pro-UK), have been studied as thrombolytic agents [67,68]. In general, these agents are administered *via* intravenous injection, and their limitations include fast clearance, lack of resistance to re-occlusion, bleeding complications and other adverse effects [67].

EFEs have significant fibrinolytic efficacy *in vitro*, showing little toxic and some side effects, for instance hemorrhage complication, during orally experiments both in animals and clinics. EFE functions in the mechanism of both fibrinolytic enzyme and plasmin-activator, distinct from all those enzymes (UK, t-PA etc.) [54-56]. It does not only degrade fibrinogen, elastin and fibrin directly, but also partially converts PLg into active plasmin. A distinct amelioration is observed in treatment of blood-high-viscosity-syndrome and thrombocytosis [69]. Nevertheless, it has chance to degrade other proteins in the presence of blood, on the basis of its lower substrate-specificity. In animal experiments, the purified enzyme, for instance F-II, often leads to hemorrhage complications when it is intravenously injected. This could be an important reason that the injection agent has not been successfully produced, but a mixture of the whole isomers made into an oral capsule in clinical treatment, instead [70]. So far, in China, Korea and Japan, EFEs have been used as a novel orally administered fibrinolytic agent to prevent and treat clotting diseases, such as acute myocardial infarction (AMI) and cerebral thrombus [71]. EFEs are stable during a long-term storage at room temperature [72], after they made into oral-capsule. Earthworm, the starting material of EFE, can be easily raised, making EFE into a relatively inexpensive thrombolytic agent applicable for production.

## Oral Administration

Biological membranes are usually not permeable to macromolecules including most enzymes, and distribution of enzymes in human body is regionally limited. Theoretically, it is difficult for them to arrive at certain targeting area through the blood-brain barrier, which has been paid attention in the research of therapeutic enzyme. It is still unclear whether the active substances are absorbed in sufficient quantities, or they are present in the blood stream for enough effect time, or they may interact

with blood components and form some complexes. Each of these possibilities may give rise to several fatal questions in the case of proteases administration. However, some therapeutic enzymes have their specific properties. They can be absorbed before degraded in the alimentary tract. Investigations on the absorption of the undegraded dietary proteins, such as β-lactoglobulin, hepatitis-B surface antigen, bromelain and epoxy-β-carotenes [73], showed that the macromolecules could be transported from the intestinal wall into blood as full size.

The history of utilization of the earthworm as a therapeutic drug source for various diseases extends back for several thousand years in China and other parts of the Far East. However, practical pharmacological studies have not been performed except on lumbrofebrin as an antifebrile. The lyophilized powder of the earthworm *L. rubellus* is administered orally to rats and dogs and its fibrinolytic and antithrombotic effects are investigated [1]. Experiments in an arteriole-venous shunt model of rats show that the thrombus weight decreased significantly [74]. These results support that earthworm powder is valuable for prevention and/or treatment of thrombosis conditions.

EFE is demonstrated to transport into blood through intestinal epithelium and to exhibit its biological function in part in circulation [8]. The immunological results show 15% intact F-III-1 is absorbed through intestinal epithelium *in vitro* with the incubation chamber method. Furthermore, after intraperitoneal injection of rats, the immunoreactively intact F-III-1 in blood is detected and about 10% full-size enzyme is transported through the peritoneum [8]. The maximum activity (30% of native F-III-1) in blood can be assayed around 60 min by chromozym TH after the injection. The N-terminal sequencing indicates that F-III-1 and -III-2 are in some similar characteristics to trypsin [75], protein transduction domain (PTD) [76], bromelain [77] and HRP [8] that have been known as transportable proteins. The sequences are rich in hydrophobic amino acid residues, which may play an important role during the membrane transportation.

## Antitumor

The extract of earthworm can not only kill the cancer cells directly *in vitro* [78,79], but also inhibit the occurrence and development of cancer *in vivo* [23,80]. EFE dissolves the fibrin around cancer cells when it is applied mutually with antitumor drugs. It helps antitumor drug to arrive at cancer, enhancing the drug's effect on the cells. It possibly becomes as a candidate to research the accessory antitumor therapeutic agent [18].

A new glycosylated component is related to apoptosis of tumor cells, which is found in *E. fetida* by Xie *et al.* [81] recently, and is highly homologous to EFEs and some serine proteases, such as F-I-1, -I-2, -II,

and EFEa. The anti-tumor activity of the glycosylated component is identified and shown by phase-contrast microscopy observation of apoptotic cells and the localization of fluorescent antibodies in cell nucleus.

U-PA is secreted by the most malignant tumors. As a response to u-PA synthesis, surrounding cells synthesize inhibitors of plasminogen activators for tissue protection, resulting in a high concentration of fibrin locally. G-90, isolated from the homogenate of *E. fetida*, containing glycolipoproteins, acts as a plasminogen activator [19-22], which is assayed with a euglobulinic mixture applied to fibrin clot from blood plasma of patients suffered from malignant tumors. The effect of G-90 on the fibrinolysis rate is not only related to its concentration, but also to histological type where the malignant tumors invade.

An antitumor bioactivity glycoprotein is also purified from earthworm (*E. fetida*) recently [82]. It is assayed by using a liver tumor model ($H_{22}$) of mice; the results show that significant antitumor effects and the life-prolonged rate was 65.4% in cultured $H_{22}$ of mice [82]. Furthermore, it has been proved that EFE can enhance the curative effects by both radiation therapy and chemotherapy [83,84].

## Assistant to Implantation

The formation of thrombus is one of the most frequent complications of introducing an artificial organ into a living body. Although many approaches to improving the blood compatibility to biomaterial have been tried, the results, so far, are not completely satisfactory.

In 1994, Ryu *et al.* [85] immobilized EFE to polyurethane using maleic anhydride methylvinyl ether copolymer (MAMEC) as an enzyme carrier. The EFE-immobilized polyurethane surface was highly antithrombogenic and had potential for reducing surface induced thrombus.

EFE-immobilized surface may minimize platelet adhesion and activation by preventing fibrinogen from adsorption or by altering the conformation of adsorbed fibrinogen at an early stage of blood contact. The antithrombogenicity increases hydrophilicity of surface on behalf of MAMEC for implantation. Clinical applications of this material to artificial organs could be expected to develop in the near future.

EFE is immobilized in a Korean type total artificial heart valve by photoreaction; polyallylamine is used as a photoreactive linker [86-88]. The proteolytic activity of the treated valves is three times higher than that of untreated valves using the azocasein method. These data show that an EFE-treated polyurethane valve leads to decreasing thrombus formation *in vivo* and that their biocompatibility is therefore greater than that of untreated valve. It is expected that EFE could be applied as a novel accessory in the artificial organs implanted into human body.

## PROBLEMS AND POTENTIAL SOLUTIONS OF EFE AS A MEDICINE

An ideal fibrinolytic medicine should have the following properties [62]: (1) Strong fibrinolytic activity; (2) Specificity on fibrinogen and fibrin; (3) Low immunogenicity; (4) Long half-life *in vivo*, (5) Low re-occlusion rate and (6) Reasonable cost.

Even though EFE has some advantages in pharmaceutical application, there are some of problems as a clinical fibrinolytic medicine. EFE directly dissolves fibrinogen and fibrin, probably producing plasmin complications. However, it hydrolyzes some other proteins *in vivo* and the broad specificity may lead to hemorrhage. The half-life of EFE should be prolonged in circulation. Recently pharmacological and clinical studies have focused on improving the efficacy and tolerability of thrombolytic agents. The potential solutions to current problems in the clinical application of EFE include: (1) improving its specificity on fibrin and decreasing the side effects; (2) enhancing the resistance to plasminogen activator inhibitor (PAI)-1 which inhibits t-PA irreversibly; (3) elongating its half-life; (4) accelerating the time course of dissolution of thrombus, preventing the recurrence of blood clotting, and (5) reducing the adverse effects resulted from hemorrhage.

# PROSPECT

### Parental Routes of Administration

The oral administration of EFE has a relatively slow pharmacological efficacy; hence it is not suitable for treatment of emergency of thrombus. If EFE were made into injection agent, it would be meaningful to treat AMI, acute cerebral thrombosis, cerebrovascular thrombus, peripheral limbs arteriovenous thrombus and other acute diseases involved in thrombosises.

Further researches could focus on the followings [89]: (1) to analyze all primary structures of EFE isozymes and identify essential groups; (2) to search the relationship between structure and function that are related to the preparation of injection agent; (3) to investigate the antigenic features of the isozymes [90]; (4) to modify chemically the structure of EFE molecule, so that those structures or groups, leading to hostile responses, are removed or blocked; and finally (5) to purify and separate the genes of EFE and to investigate whether or not EFE could be made into an injection agent by gene engineering.

## Gene Expression

Although some of EFE genes are being expressed [25,43], there have been no recombinant EFEs available as a medicine made by gene expression. The medicine is hard to produce by genetic engineering, because (1) The protein expressed in *E. coli.* trends to misfold and thus readily form inclusion body, which has little biological activity; (2) The isozyme-complex extracted from earthworm may have stronger clinical effect than one isozyme expressed by a single gene. On the other hand, so far, earthworm is raised in copious amounts, providing a sufficient source to prepare the enzymes.

So far, a few of isozymes (F-III-1, III-2 and -II, etc) have been purified and studied in detail. Some molecular and pharmacological properties of EFEs are still unclear. However, EFEs are usefully investigated as a candidate to therapies for diabetes, anti-fat crystallization, blood pressure adjuster (hypertension and hypotension), solvent thrombus, cerebral vessels, angina, liver disorder, cataracts, glaucoma, varicosity, rheumatism, bedsores, nephritis, vibration disease, etc [91-100]. These would be meaningful topics for further studies.

## ABBREVIATIONS

| | | |
|---|---|---|
| EFE | = | Earthworm fibrinolytic enzyme |
| PLg | = | Plasminogen |
| LK | = | Lumbrokinase |
| HPLC | = | High pressure liquid chromatography |
| EPE | = | Earthworm powder enzyme |
| e-TPA | = | Earthworm-tissue plasminogen activator |
| e-PA | = | Earthworm plasminogen activator |
| EFEa | = | Component A of EFE |
| SDS-PAGE | = | Sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| PCR | = | Polymerase chain reaction |
| RT-PCR | = | Reverse transcriptase-PCR |
| PPE | = | Pocine pancreatic elastase |
| HLE | = | Human leukocyte elastase |
| MIR | = | Multiple isomorphous replacement |
| DFP | = | Diisopropyl fluorophosphase |
| SBTI | = | Soybean trypsin inhibitor |
| HD | = | 1, 6-hexamethylendiamine |
| TPCK | = | Tosyl-phenylalanyl chloromethylketose |
| TLCK | = | Tosyl-lysyl-chloromethylketose |

| | | |
|---|---|---|
| ε-ACA | = | ε-amino caproic acid |
| EDTA | = | Ethylene diamine tetraacetic acid |
| $\alpha_2$M | = | $\alpha_2$-Macroglobulin |
| t-PA | = | Tissue plasminogen activator |
| UK | = | Urokinase |
| BSA | = | Bovine serum albumin |
| CK | = | Creatine kinase |
| IgG | = | Immunoglobulin G |
| AMI | = | Acute myocardial infarction |
| SK | = | Streptokinase |
| rt-PA | = | Recombinant tissue-type plasminogen activator |
| SAK | = | Staphylokinase |
| pro-UK | = | Recombinant prourokinase |
| HRP | = | Horseradish peroxidase |
| PTD | = | protein transduction domain |
| u-PA | = | U-plasminogen activator |
| MAMEC | = | Maleic anhydride methylvinyl ether copolymer |
| PAI | = | Plasminogen activator inhibitor |

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## REFERENCES

[1] Mihara, H.; Sumi, H.; Mizumoto, H.;. In *Recent advances in Thrombosis and Fibrinolysis;* Academic Press, Tanaka K., Ed.; New York, **1990**; pp.287-298.,

[2] Keilin, D.; *Q. J. Microsc. Sci.*, **1920**, *65*, 33-61.

[3] Mihara, H.; Sumi, H.; Yoneta, T.; Mizumoto, H.; Ikeda, R.; Seiki, M.; Maruyama, M.; *J. Physiol*, **1991**, *41*, 461-472.

[4] Mihara, H.; Sumi, H.; Akazawa, K.; *Tromb. Haemostas*, **1983**, *50*, 258.

[5] Zhou, Y.C.; Zhu, H.; Chen, Y.C.; *Acta Boochem. et Biophys. Sin.*, **1988**, *20*, 35-42.

[6] Lu, Y.H.; Jin, R.C.; Wu, Y.W.; *Chin. Biochem. J.*, **1988**, *4*, 166-172.

[7] Zhou, J.; Fan, Q.; Wu, C.; He, R.Q.; *Prot. and Pept. Lett.*, **1997**, *4*, 409-414.

[8] Fan, Q.; Wu, C.; Li, L.; Fan, R.; Wu, C.; Hou, Q.M.; He, R.Q.; *Biochim. et Biophys. Acta*, **2001**, *1526*, 286-292.

[9] Wu, P.; Fan, R.; *Acta Biophys. Sin.*, **1986**, *2*

[10] Yang, J.S.; Ru, B.G.; *Comp. Biochem. Physiol.*, **1997**, *118B*, 623-631.

[11] Yang, J.S.; Li, L.Y.; Ru, B.G.; *Chinese J. Biochem. Mol. Biol.*, **1998**, *14*, 156-163.

[12] Yang, J.S.; Li, L.Y.; Ru, B.G.; *Chin. J. Biochem. Mol. Biol.*, **1998**, *14* , 164-169.

[13] Yang, J.S.; Li, L.Y.; Ru, B.G.; *Chin. J. Biochem. Mol. Biol.*, **1998**, *14* , 412-416.
[14] Yang, J.S.; Li, L.Y.; Ru, B.G.; *Chin. J. Biochem. Mol. Biol.*, **1998**, *14* , 417-421.
[15] Yang, J.S.; Guo, Y.Q.; Ru, B.G.; *Chin. J. Biochem. Mol. Biol.*, **1998**, *14*, 721-725.
[16] Tang, Y.; Zhang, J.; Gui, L.L.; Wu, C.; Fan, R.; Chang, W.R.; Liang, D.C.; *Acta Cryst.*, **2000**, *56*, 1659-1661.
[17] Tang, Y.; Liang, D.C.; Jiang, T.; Zhang, J.P.; Gui, L.L.; Chang, W.R. *J. Mol. Biol.*, **2002**, *321*, 57-68.
[18] Popovic, M.; Hrcenjak, T.M.; Babic, T.; Kos, J.; Grdisa, M.; *Pathol. Oncol. Res.*, **2001**, *7*, 197-202.
[19] Hrženjak, T.M.; Popović, M.; Tiška-Rudman, L.; *Pathol. Oncol. Res.*, **1998**, *4*, 206-211.
[20] Hrženjak, T.; Popović, M.; Božić, T.; Grdisa, M.; Kobrehel, D.; Tiška-Rudman, L.; *Comp. Biochem. Phys. B*, **1998**, *119*, 825-832.
[21] Grdisa, M.; Popović, M.; Hrženjak, T.; *Comp. Biochem.. Physiol. A Mol. Integr. Physiol.*, **2001**, *128*, 821-825.
[22] Popović, M.; Hrženjak, T.; Grdisa, M.; Vukovic, S.; *Gen. Pharmacol.*, **1998**, *30*, 795-800.
[23] Hrženjak, T.; Hrženjak, M.; Kasuba, V.; Efenberger-Marinculic, P.; Levanat, S.; *Comp. Biochem. Physiol.*, **1992**, *102*, 441-447.
[24] Nakajima, N.; Mihara, H.; Sumi, H.; *Biosci. Biotech. Biochem.*, **1993**, *57*, 1726-1730.
[25] Xu, Y.H.; Lian, G.D.; Sun, Z.J.; Chen, F.; Fu, S.H.; Chai, Y.B.; Hou, Y.D.; *Prog. Biochem. Biophys.*, **2002**, *29*, 610-614.
[26] *GenBank database*, accession number, AF 109648.
[27] http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi
[28] Nakajima, N.; Mihara, H.; Sumi, H.; *Biosci. Biotech. Biochem.*, **1999**, *63*, 2031-2033.
[29] Zhao, X.Y.; Jing, T.Y.; *Chin. J. Biochem. Mol. Biol.*, **1998**, *14*, 407-411.
[30] Zhao, X.Y.; Jing, T.Y.; *Prog. Biochem. Biophys.*, **2001**, *28*, 218-220.
[31] Li, L.; Zhao, J.; He, R.Q.; *Prot. and pept. Lett.*, **2003**, *10*, 91-98.
[32] *GenBank database*, accession number, BAB40768.1
[33] *GenBank database*, accession number, BAB40767.1
[34] *GenBank database*, accession number, AAM73677.1
[35] *GenBank database*, accession number, AAL28118.1
[36] Takahashi, L.H.; Radhakrishnan, R.; Rosenfiedld, R.E.; Meyer, E.F.; Trainor, D.A.; *J. Am. Chem. Soc.*, **1989**, *111*, 3368-3374.
[37] Wei, A.Z.; Mayr, I.; Bode, W.; *FEBS Lett.*, **1988**, *234*, 367-373.
[38] Marquart, M.; Walter, J.; Deisenhofer, J.; Bode, W.; Huber, R.; *Acta Crystallog.*, Sect. B, **1983**, *39*, 480-490.
[39] Mac Sweeney, A.; Birrane, G.; Walsh, M.A.; O'connell, T.; Malthouse, J.P.; Higgins, T.M.; *Acta Crystallog.*, Sect. D, **2000**, *56*, 280-286.
[40] Spraggon, G.; Phillips, C.; Nowak, U.K.; Ponting, C.P.; Saunders, D.; Dobson, C.M.; Stuart, D.I.; Jones, E.Y.; *Structure*, **1995**, *3*, 681-691.
[41] Lamba, D.; Bauer, M.; Huber, R.; Fischer, S.; Rudolph, R.; Kohnert, U.; Bode, W.; *J.*

*Mol. Biol.*, **1996**, *258*, 117-135.
[42] Wang, X.Q.; Lin, X.L.; Lou, J.A.; Tang, J.; Zhang, X.J.; *Science*, **1998**, *281*, 1662-1665.
[43] Sugimoto, M.; Nakajima, N.; *Biosci. Biotechnol. Biochem.*, **2001**, *65*, 1575-1580.
[44] *GenBank database*, accession number, CAA11132.
[45] *GenBank database*, accession number, CAA64472.
[46] Sumi, N.; Nakajima, N.; Michara, H.; *Comp. Biochem. Physiol.*, **1993**, *106*, 763-766.
[47] Astrup, T.; Mullertz, S.; *Arch. Biochem. Biophys.*, **1952**, *40*, 346-359.
[48] Zhou, J.; Jiang, K.; He, R.Q.; Han, Y.M.; *Acta Biophys. Sinica*, **1997**, *13*, 531-535.
[49] Mao, S.Z.; Yan, Z.; Chen, S.G.; *J. Wenzhou Medical College*, **2000**, *30*, 277-278.
[50] Wu, C.; Li, L.; Zhao, J.; Fan, Q.; He, R.Q.; *Inter. J. Biol. Macromol.*, **2003**, *In pressing.*
[51] Jensen, P.E.; Sottrup-Jensen, L.; *J. Bio. Chem.*, **1986**, *261*, 15863-15869.
[52] Barret, A.J.; Starkey, P.M.; *Biochem. J.* **1973**, *133*, 709-724.
[53] Wu, X.Q.; Wu, C.; He, R.Q.; *Prot. and Pept. Lett.*, **2002**, *9*: 75-80.
[54] Kasai, S.; Arimura, H.; Nishida, M.; Suyama, T.; *J. Biol. Chem.*, **1985**, *260*, 12377-12381.
[55] Madison, E.L.; Coombs, G.S.; Corey, D.R.; *J. Biol. Chem.*, **1995**, *270*, 7558-7562.
[56] Kim, J.S.; Kang, J.K.; Chang, H.C.; Lee, M.; Kim, G.S.; Lee, D.K.; Kim, S.T.; Kim, M.; Park, S.; *J. Korean Med. Sci.*, **1993**, *8*, 117-120.
[57] Matsuo, O.; Rijken, D.C.; Collen, D.; *Nature*, **1981**, *291*, 590-591.
[58] Bach-Gansmo, E.T.; Halvorsen, S.; Godal, H.C.; Skjonsberg, O.H.; *Thromb Res.*, **1994**, *73*: 61-68.
[59] Lin, S.Q.; Yu P.; Lan, R.F.; *Pharmaceutical Biotechn.*, **2000**, *7*, 229-233.
[60] Zhang, G.P.; Jin, H.M.; Zhang, M.; Cao, X.; Qian, R.Z.; Yang, S.C.; *Chin. J. Geriatrics*, **1998**, *6*.
[61] Zhang, G.P.; Qian, R.Z.; Yang, S.C.; Jin, H.M.; *National Med. J. Chin.*, **1998**, *78*.
[62] Collen, D.; *Thromb. Haemost.*, **1980**, *43*, 77-89.
[63] Mihara, H.; Maruyama, M.; Sumi, H.; *Southeast Asian J. Trop. Med. Public Health Suppl.*, **1992**, *2*, 131-140.
[64] Hoylaerts, M.; Rijken, D.C.; Lijnen, H.R.; Collen, D.; *J. Biol. Chem.*, **1982**, *257*, 2912-2919.
[65] Wun, T.C.; Schleuning, W.D.; Reich, E.; *J. Biol. Chem.*, **1982**, *257*, 3276-3283.
[66] Standker, L.; Sillard, R.; Bensch, K.W.; Ruf, A.; Raida, M.; Schulz-Knappe, P.; Schepky, A.G.; Patscheke, H.; Forssmann, W.G.; *Biochem. Biophys. Res. Commun.*, **1995**, *215*, 896-902.
[67] Verstraete, M.; *Am. J. Med.*, **2000**, *109*, 52-58.
[68] Zhao, J.; Li, D.F.; *Microbiology*, **2002**, *29*, 102-107.
[69] Cong, Y.W.; Liu. Y.M.; Chen, J.P.; *Chin. J. Biochem. Pharm,*, **2000**, *21*, 159-162.
[70] Nakajima, N.; Ishihara, K.; Sugimoto, M.; *Biosci. Biotechn. Biochem.*, **1996**, *60*, 293-300.
[71] Jin, L.; Jin, H.; Zhang, G.; Xu, G.; *Clin Hemorheol Microcirc*, **2000**, *23*, 213-218.
[72] Nakajima, N.; Sugimoto, M.; Ishihara, K.; *J. Biosci. Bioengi.*, **2000**, *90*, 174-179.

[73] Barua, A.B.; *Biochem. J.*, **1999**, 15, *339*, 359-362 .
[74] Vilhardt, H.; Lundin, S.; *Acta. Physiol. Scand.*, **1986**, *126*, 601-607.
[75] Mikes, O.; Holeysovesky, V.; Thmasek, V.; Sorm, F.; *Biochem. Biophys. Res. Commun.*, **1966**, *24*, 346-352.
[76] Green, M.; Loewenstein, P.M.; *Cell*, **1988**, *55*, 1179-1188.
[77] *GenBank database*, accession number, D14058.
[78] Zhang, F.; Wang, K.W.; *J.4th Mili. Med. Uni.*, **1987**, *8*, 224.
[79] Zeng, X.L.; Zhang, B.Y.; Mai, X.X.; *J. Shanxi Medi. Col.*, **1995**, *26*, 81-83.
[80] Wang, K.W.; Zhang, S.Z.; Li, Y.R.; *J. 4th Mili. Med. Uni.*, **1986**, *7*, 85-88.
[81] Xie, J.B.; Guo, Z.Q.; Weng, N.; Wang, H.T.; Jiang, G.Q.; Ru, B.G.; *Prog. Biophys. Biochem.*, **2003**, *In pressing.*
[82] Lin, S.Q.; Yu P.; Lan, R.F.; Cheng, W.; Huan Y.D.; *Strait Pharm. J.*, **2000**, *12*, 59-61.
[83] Zhang, WL.; Li, L.Q.; Mao, H.S.; *Chin. J. of Clin. Oncol.*, **1991**, *18*, 181-182.
[84] Zhang, S.Z,; Tian, Q.; Wang, K.W.; *J. 4th Mili. Med. Uni.* **1992**, *13*, 165-168.
[85] Ryu, G.H.; Park, S.; Kim, M.; *J Biomed Mater Res.*, **1994**, *28*, 1069-1077.
[86] Park, Y.; Ryu, E.; Kim, H.; *Artif Organs.*, **1999**, *23*, 210-214.
[87] Gly, H.R.; Dong, K.H.; Seonyang, P.; *J. Biom. Materials Res.*, **1995**, *29*, 403-409.
[88] Ryu, G.H.; Park, S.; Han, D.K.; Kim, Y.H.; Min, B.; *ASAIO J.*, **1993**, *39*, M314-318.
[89] Wang, Z.W.; Liang, X.W.; Fan, X.Y.; *Chin. Trad. Gerbal Drugs*, **2000**, *31*, 386-389.
[90] Zhao, X.Y.; Jing, T.Y.; Jiang, X.Y.; Duan, M.X.; Zheng, C.X.; *Chin. J. Biochem and Mol. Biol.*, **2002**, *18*, 209-212.
[91] Roch, P.; Valembois, P.; Davant, N.; Lassegues, M.; *Comp. Biochem. Physiol.*, **1981**, *698*, 829-836.
[92] Roch, P.; Lassegues, M.; Valembois, P.; *Dev. Comp. Immunol.*, **1991**, *15*, 27-32.
[93] Valembois, P.; Roch, P.; Lassegues, M.; In *Antibacterial molecules in Annelids. Immunity in Invertebrates*; Brehelin, M.; Springer Verlag, Ed.; Berlin, **1986**; pp. 14-93.
[94] Hrzenjak, M.; Kobrehel, D.; Levanat, S.; Jurin, M.; Hrzenjak, T.; *Comp. Biochem. Physiol. B*, **1993**, *104*, 723-729.
[95] Kauschke, E.; Pagliara P.; Stabili, L.; Cooper E.L.; *Comp. Biochem. Physiol. B*, **1997**, *116*, 235-242.
[96] Eue, I.; Kauschke, E.; Mohrig, W.; Copper, E.L.; *Devel. and Comp. Immun.*, **1998**, *22*, 13-25.
[97] Milochau, A.; Lassegues, M.; Valembois, P.; *Biochim Biophys Acta*, **1997**, *1337*, 123-132.
[98] Lange, S.; Kauschke, E.; Mohrig, W.; Cooper E.L.; *Eur. J. Biochem.*, **1999**, *262*, 547-556.
[99] Beschin, A.; Bilej, M.; Brys, L.; Torreele, E.; Lucas, R.; Magez, S.; De Baetselier P.; *Nature*, **1999**, *400*, 627-628.
[100] Hwang, C.M.; Kim, D.I.; Huh, S.H.; Min, B.G.; Park, J.H.; Han, J.S.; Lee, B.B.; Kim, Y.I., Ryu, E.S.; Kim, J.W.; *J. Cardiovasc. Surg.*, **2002**, *43*, 891-894.

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